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

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J. Econ. Entomol. 98(2): 534–551 (2005)

ABSTRACT Western corn rootworm, *Diabrotica virgifera virgifera* LeConte, has overcome crop rotation in several areas of the north central United States. The effectiveness of crop rotation for management of corn rootworm has begun to fail in many areas of the midwestern United States, thus new management strategies need to be developed to control rotation-resistant populations. Transgenic corn, *Zea mays* L., effective against western corn rootworm, may be the most effective new technology for control of this pest in areas with or without populations adapted to crop rotation. We expanded a simulation model of the population dynamics and genetics of the western corn rootworm for a landscape of corn; soybean, *Glycine max* (L.); and other crops to study the simultaneous development of resistance to both crop rotation and transgenic corn. Results indicate that planting transgenic corn to first-year cornfields is a robust strategy to prevent resistance to both crop rotation and transgenic corn in areas where rotation-resistant populations are currently a problem or may be a problem in the future. In these areas, planting transgenic corn only in continuous cornfields is not an effective strategy to prevent resistance to either trait. In areas without rotation-resistant populations, gene expression of the allele for resistance to transgenic corn, R, is the most important factor affecting the evolution of resistance. If R is recessive, resistance can be delayed longer than 15 yr. If R is dominant, resistance may be difficult to prevent. In a sensitivity analysis, results indicate that density dependence, rotational level in the landscape, and initial allele frequency are the three most important factors affecting the results.

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

WESTERN CORN ROOTWORM, *Diabrotica virgifera virgifera* Leconte, is the most serious insect pest of continuously grown corn, *Zea mays* L., in the United States (Levine and Oloumi-Sadeghi 1991). Adults of *D. virgifera virgifera* typically are present in cornfields from July through frost, where they feed on corn foliage, silks, pollen, and immature kernels. Larval feeding on corn roots can cause yield loss by reducing water uptake and nutrient absorption, facilitating the entry of stalk- and root-infesting microorganisms, and making plants susceptible to lodging (falling over) (Levine and Oloumi-Sadeghi 1991).

During late summer, eggs of *D. virgifera virgifera* are normally laid in cornfields where they overwinter; few eggs are deposited in other crops. The larvae, which emerge during the spring, can survive only on the roots of corn and on a limited number of grass species (Levine and Oloumi-Sadeghi 1991, Clark and Hibbard, 2004, Oyediran et al. 2004). For many years, corn producers throughout the north central United States have managed this pest by practicing crop rotation, planting corn and a nonhost crop [such as soybean, *Glycine max* (L.)] in alternate years. This has been effective because eggs lain in a rotated cornfield will hatch the next year in the nonhost crop and the larvae will not survive.

However, recent investigations have indicated that the newly observed tendency of adults to move out of their natal cornfield into non-corn areas, where they lay eggs, is a behavioral change that allows this insect to overcome management by crop rotation (Onstad et al. 2003a, Rondon and Gray 2004). These rotation-resistant "variant" populations have spread throughout much of the Illinois and Indiana over the past 10–15 yr, reaching into western Ohio and southern Michigan (Onstad et al. 1999, 2003b).

A model by Onstad et al. (2003a) indicated that transgenic corn effective against corn rootworm may be a valuable new technology to control rotation-resistant populations. However, this model did not consider many of the complexities involved with the

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0022-0493/05/0534–0551$04.00/0

use of transgenic crops, including the potential for resistance. For ≈10 yr, scientists have focused on 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). Much of the emphasis has been on the high dose/refuge strategy for which industry scientists attempt to create plants that can express toxins at doses high enough to kill most if not all heterozygotes (SR) that may be partially resistant to the toxin at lower doses (Gould 1998, Caprio 2001). The goal is functional dominance of susceptibility to the toxin. The refuge is used to produce susceptible homozygotes (SS) to mate with any surviving resistant individuals to produce only SS or SR offspring that can be killed by the transgenic crop (Shelton et al. 2000, Tang et al. 2001).

No current models focus on the simultaneous evolution of resistance to both crop rotation and transgenic crops. Onstad et al. (2001a) created a model 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 with the high dose/refuge strategy. However, this model assumed that transgenic crops were deployed only in continuous cornfields and did not analyze the impact of rotation-resistant phenotypes. Similarly, Storer (2003) created a spatially explicit model that simulated adaptation to transgenic maize but did not consider the influence of rotation-resistant phenotypes. In this article and a companion article (Crowder and Onstad 2005), we expand both models by Onstad et al. (2001a, 2003a) to evaluate the risk of resistance by western corn rootworm to both transgenic crops and crop rotation in areas with or without rotation-resistant phenotypes. In this article, our analysis is focused on the expanded daily time-step model of Onstad et al. (2001a).

The first commercial transgenic product for control of the western corn rootworm was commercialized in 2003, so research on the interaction between transgenic crops and corn rootworm is a high priority. Expanding models to simulate both rotation resistance and resistance to transgenic crops should provide management advice to growers who want to use transgenic crops in the future and guide further research on transgenic crops. We investigate how the dose of the toxin in the crop, gene expression, and different refuge strategies affect the development of resistance.

Our analysis is focused on several areas. First, we investigated the evolution of resistance to crop rotation without the use of transgenic corn. Second, we simulated the use of transgenic corn in rotated cornfields, continuous cornfields, or both to study the most effective strategies to slow resistance to crop rotation and prevent resistance to transgenic corn. In this analysis, we also analyzed whether crop rotation can affect resistance to transgenic corn and vice versa. Third, we investigated the use of transgenic corn in areas without rotation-resistant phenotypes.

Materials and Methods

In this section, we describe the creation of the model and its analysis. The model was adapted from the deterministic daily time-step model of Onstad et al. (2001a). Unless otherwise noted, the details and assumptions used to create the functions in the model are explained in Onstad et al. (2001a). First, we describe observations and literature that support the ecological framework and biological processes used to create the model. Second, we describe the ecological equations used to develop the model. Each of the ecological equations was calculated with a daily time-step. Third, we discuss the standard simulation conditions for areas with and without rotation-resistant phenotypes. Then, we describe the sensitivity analyses we performed.

The state variables for the beetles are both space- and age-structured. Male and unmated female beetles are distinguished by three characteristics: genotype, g; field, f; and age, t. Mated female beetles are identified by four characteristics: genotype; field; age; and genotype of mate, m. Immature beetles are identified by genotype and field. All indices, subscripts, parameters, and state variables are defined in Appendix 1.

Population Genetics. We assumed a simple population genetics model of *D. virgifera virgifera* to explain the evolution 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 genetic system was chosen because it is comparable with several natural systems described by Onstad et al. (2001b) and has been used in models that simulated the development of resistance by western corn rootworm to crop rotation (Onstad et al. 2001b, 2003a) 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 regards to crop rotation, we defined the X allele for no movement out of corn and the Y allele for the tendency to move to all patches. We studied three types of gene expression for each trait: Y as recessive, additive, and dominant and R as recessive, partially recessive, and dominant. We assumed 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 planted after soybean in a 2-yr rotation (rotated corn), C_r; soybean, which precedes corn in a 2-yr rotation, Soy; and the extra noncorn, Ex. We assumed 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 corn patches that are planted to a transgenic cultivar is Tc and Tr, respectively. In cases where Tc or Tr >0, we studied refuge sizes occupying 5, 10, 20, or 30% of the field.

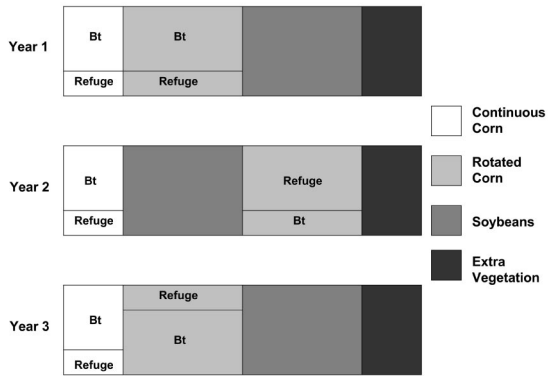

Fig. 1. Typical model landscape over a 3-yr time horizon with four crops and transgenic corn planted to both continuous and rotated cornfields.

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 assumed that refuge and transgenic fields remain at the same site in successive years. In simulations with transgenic corn planted to the rotated cornfield, the location of the refuge in the rotated cornfield is 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. Figure 1 is meant to show a region with the maximum six fields but should not suggest that this is a spatial model with a region having absolute dimensions.

Corn Phenology. Each simulation starts with the growth stage of corn being 4.0, which is the last vegetative stage when tassels are completely visible. The peak of growth, stage 5 (silking), occurs on Julian day 200. The five stages after silking—blister, milk, dough, dent, and maturity—occur on Julian days 212, 220, 226, 239, and 260, respectively (Ritchie et al. 1993). We used linear interpolation between peaks to calculate the decimal stages of corn phenology. Once stage 10 is reached, the corn stage remains at 10 for the rest of the season. We assume that the corn landscape in the model is uniform with respect to plant maturity.

Insect Phenology. The immature stages include eggs, larvae, and pupae. The maturation of immatures is not modeled explicitly. We assume that adult beetles of any genotype mature at the same rate. Males and females have a 37-d emergence period centered on days 207 and 215, respectively (Onstad et al. 2001a).

Adult males have two life stages: teneral and nonteneral. Adult females have three life stages: teneral, preovipositing, and ovipositing. Onstad et al. (2001a) allowed a proportion of females to remate and included two additional life stages: a second nonovipositing and ovipositing period. However, they observed that remating did not occur because all males died before the earliest emerging females had a chance to remate. Therefore, we did not allow females to remate in this model and did not include the second nonovipositing or ovipositing period.

The teneral stage lasts 1 and 2 d for females and males, respectively (Onstad et al. 2001a). Males may mate during the 42 d after the teneral stage (Quiring and Timmins 1990). We assume that age of males does not determine mating success (Branson et al. 1977). For females, the preoviposition period lasts 12 d (Branson and Johnson 1973, Hill 1975). Under normal conditions, females mate during the preoviposition period and lay eggs for 60 d during the oviposition period. In the model males and females can live for a maximum of 44 and 73 d, respectively.

Adult Survival. We model the development and survival of adults independently. Three factors determine adult survival in the model: first date of 0°C in the fall, nutritional status of corn, and age of the adult. All adults die on 19 October, or Julian day 292 in the model, which is the average first date of 0°C in Champaign, IL, each year (Onstad et al. 2001a).

The daily survival rate based on nutrition, Snut, declines after growth stage 5.5 of the corn (Onstad et al. 2001a) and is the same for females and males. We do not model age-specific mortality for males (Onstad et al. 2001a). The age of females becomes an important factor after 6 wk (Elliott et al. 1990a, b). The survival of females based on age, Sage, is 1.0 for the first 42 d. For females after day 42, the daily survival rate $\mathrm{Sage}(t) = 0.98$ (Onstad et al. 2001a).

The proportions of females and males surviving each day are S_f and S_m, respectively. S_f and S_m are calculated as:

$$S_f(t,g,f) = \mathrm{Sage}(t) \times \mathrm{Snut} \quad \text{for } 1 \leq t \leq 73 \qquad [1]$$

$$S_m(t,g,f) = \mathrm{Snut} \quad \text{for } 1 \leq t \leq 44 \qquad [2]$$

For females, $S_f(t,g,f) = 0$ for $t > 73$, and for males, $S_m(t,g,f) = 0$ for $t > 44$. Similarly, $S_f(t,g,f) = S_m(t,g,f) = 0$ on Julian day 292.

Adult Dispersal. The following assumptions about adult dispersal are included in the model. Movement within a field is random (Naranjo 1994). Oviposition status does not affect dispersal by females among fields (Naranjo 1990a, b). Because we did not model variation in planting date of corn or phenology between fields, we assume that dispersal is not affected by the growth stage of corn because all corn crops in the landscape are phenologically equivalent (Onstad et al. 2001a).

We assume that teneral females and males, as well as all other unmated females, do not move outside of the natal field (Onstad et al. 2001a). The dispersal of all other adults is affected by the alleles at the locus for resistance to crop rotation. We assume that homozygous (–XX) individuals move only to cornfields, whereas homozygous (–YY) individuals move to all fields. The movement of the heterozygotes (–XY) is influenced by the gene expression at the locus for resistance to crop rotation.

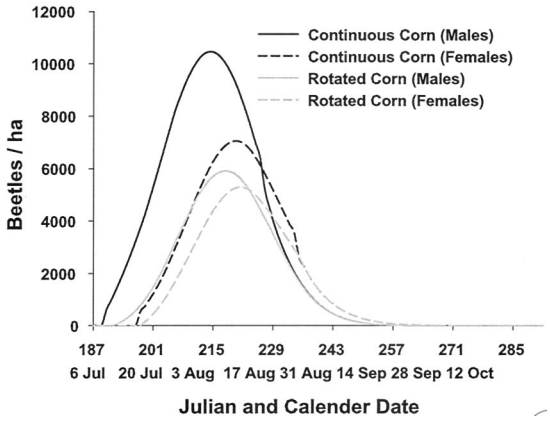

Fig. 2. Simulated densities of male and female adults (beetles/ha) over one season. The amount of land planted to $C_c = C_r = 0.5$, with an initial adult density of 50,000/ha initialized in the continuous corn. The initial transgenic resistant (R) and rotation-resistant (Y) allele frequencies are 0.

Females of the mutant phenotype disperse away from corn more than males do (Rondon and Gray 2003). To simulate this, a proportion, p, of males remain in the natal cornfield. The standard value is p = 0.75, based on the model of Onstad et al. (2001a) and data of Godfrey and Turpin (1983). The others (1 − p) move out of the natal field and are distributed throughout the rest of the region.

Onstad et al. (2001a) assumed that 2 and 0.5% of females and males, respectively, moved outside of the natal field each day. However, in preliminary simulations of this model, this dispersal pattern maintained too many adults within continuous cornfields for rotation-resistance to develop even at high levels of crop rotation (>95%). This is unrealistic because rotation resistance has evolved at these levels of crop rotation within 16 yr in Illinois since the first invasion by the western corn rootworm in the early 1970s (Onstad et al. 1999, 2003b). To better simulate this phenomenon, we used a dispersal pattern for individuals similar to that used by Onstad et al. (2001b, 2003a). We assume that movement by normal phenotypes (–XX) after mating is random throughout all cornfields within the landscape, with mated females distributed proportionally across all cornfields in the region according to the proportional area of each cornfield compared with the total area planted to corn. This pattern mimics the ultimate result of density-dependent dispersal, which cannot be modeled mechanistically because of a lack of data. This type of dispersal also results in a model that comes close to simulating a panmictic population. With this dispersal pattern, the proportion of females in first-year corn reaches 70–80% by September, which is similar to the range observed by Godfrey and Turpin (1983), O'Neal et al. (1999), and the model of Onstad et al. (2001a).

The seasonal result of this dispersal pattern is shown in Fig. 2. We simulated the model with $C_c = C_r = 0.5$, Soy = Ex = 0, 50,000 adults/ha initialized in the continuous cornfield, and the alleles for resistance set to 0. During July and early August, the number of males and females is greater in the continuous cornfield relative to the rotated field because no adults emerge in the rotated cornfield. However, dispersal by adult beetles throughout the region results in approximately the same number of males and females in both fields by mid- to late August. These results are similar to the densities of beetles in continuous and rotated cornfields observed by Godfrey and Turpin (1983).

We assumed that movement by rotation-resistant phenotypes (–YY) after mating is random throughout the landscape, with mated females being distributed proportionally across the region according to the proportional areas of the fields. Movement by heterozygotes is controlled by the expression of the Y-allele. If Y is recessive, heterozygotes move only to corn after mating. If Y is dominant, heterozygotes move randomly throughout the entire landscape after mating. In the additive case, 50% of the heterozygotes move randomly throughout corn after mating and 50% move randomly throughout all fields (Onstad et al. 2001b, 2003a).

The probability of an adult leaving field f based on the age and the genotype of the beetle is $D_f(t,g,f)$. The alleles for resistance to transgenic corn, denoted by dashes, do not affect dispersal. Alleles denoted below with capital letters are dominant, whereas alleles denoted with lowercase letters are recessive. The additive case is denoted as (–xy). For the purpose of simplifying these functions and others described later, we refer to each field by numbers (1–6). Soybean, extra vegetation, continuous corn refuge, rotated corn refuge, transgenic continuous corn, and transgenic rotated corn are fields 1, 2, 3, 4, 5, and 6, respectively. For mated females,

$$D_f(t,g,f) = 0 \quad \text{for } t < 2 \text{ and all genotypes}$$

$$D_f(t,g,f) = \left(1 - P_f \Big/ \sum_{i=3}^{6} P_i\right) \quad \text{for } t \geq 2,\ g = (-XX) \text{ or } g = (-Xy)$$

$$D_f(t,g,f) = (1 - P_f) \quad \text{for } t \geq 2,\ g = (-YY) \text{ or } g = (-xY)$$

$$D_f(t,g,f) = 0.5 \times \left[\left(1 - P_f \Big/ \sum_{i=3}^{6} P_i\right) + (1 - P_f)\right] \quad \text{for } t \geq 2,\ g = (-xy) \qquad [3]$$

For males,

$$D_m(t,g,f) = 0 \quad \text{for } t < 3 \text{ and all genotypes}$$

$$D_m(t,g,f) = \left(1 - P_f \Big/ \sum_{i=3}^{6} P_i\right) \times (1 - p)$$
$$\text{for } t \geq 3,\ g = (-XX) \text{ or } g = (-Xy)$$

$$D_m(t,g,f) = (1 - P_f) \times (1 - p)$$
$$\text{for } t \geq 3,\ g = (-YY) \text{ or } g = (-xY)$$

$$D_m(t,g,f) = 0.5 \times \left[\left(1 - P_f \Big/ \sum_{i=3}^{6} P_i\right) \times (1 - P_f)\right] \times (1 - p)$$
$$\text{for } t \geq 3,\ g = (-xy) \qquad [4]$$

P_i represents the area planted to each cornfield in the region $(3 \leq i \leq 6)$.

The proportion of dispersers of genotype g entering field f from field j each day is C(g,f,j). For the normal phenotype, only cornfields are entered, so C is the proportion of cornfield f relative to all cornfields not including field j. To calculate this, we sum over all the cornfields (P_i) and subtract the amount of area in field j.

$$C(g,f,j) = \left[P_f \Big/ \left(\left\{\sum_{i=3}^{6} P_i\right\} - P_j\right)\right]$$
$$\text{for } f \geq 3,\ g = (-XX) \text{ or } g = (-Xy)$$

$$C(g,f,j) = 0$$
$$\text{for } f < 3,\ g = (-XX) \text{ or } g = (-Xy)$$

$$C(g,f,j) = [P_f/(1 - P_j)]$$
$$\text{for all f and } g = (-YY) \text{ or } g = (-Yx) \qquad [5]$$

In the additive case (–xy), we assume that 50% of the heterozygotes enter all fields, whereas the other 50% enter only cornfields. In cases where (–xy) beetles disperse out of soybean or extra vegetation (fields 1 and 2), we assume that 50% of these adults will move back only to corn (fields 3–6), whereas the other 50% move to all fields.

$$C(g,f,j) = 0.5 \times [P_f/(1 - P_j)]$$
$$\text{for } f < 2 \text{ and all j},\ g = (-xy)$$

$$C(g,f,j) = 0.5 \times \left[P_f/(1 - P_j) + \left(P_f \Big/ \sum_{i=3}^{6} P_i\right)\right]$$
$$\text{for } f \geq 3,\ j < 3,\ g = (-xy)$$

$$C(g,f,j) = 0.5 \times \left[P_f/(1 - P_j) + \left(P_f \Big/ \left\{\left[\sum_{i=3}^{6} P_i\right] - P_j\right\}\right)\right]$$
$$\text{for f, j} \geq 3,\ g = (-xy) \qquad [6]$$

Sexual Activity. The probability of a female mating with a male of a given genotype equals the proportion of each male genotype in the female's field on that day. Because Onstad et al. (2001a) found that even the lowest levels of male dispersal were enough to result in the mating of all teneral females, we assumed that all unmated females would mate on a given day in a given field, f, if there were any nonteneral males in the field. Therefore, the proportion of unmated females in a given field I(f) was 1.0 if TM(f) > 0. The total number of unmated females, TU, and nonteneral males, TM, on a given day in a given field, f, are

$$TU(f) = \sum_{g=1}^{9} \sum_{t=1}^{73} U(t, g, f) \qquad [7]$$

$$TM(f) = \sum_{g=1}^{9} \sum_{t=3}^{44} M(t, g, f) \qquad [8]$$

where U and M are unmated females and nonteneral males, respectively.

Equations for Adult State Variables. The following equations are calculated with a daily time-step. Thus, the subscript d for day is omitted but implicit in all the state variables. In all the equations, if only one cornfield exists, then no normal (–XX) individuals emigrated. Unmated females, U, do not move. Mated females and males can live up to 73 and 44 d, respectively. For unmated females U,

$$U(t+1,g,f) = [1 - I(f)] \times S_f(t,g,f) \times U(t,g,f)$$
$$\text{for } 1 \leq t \leq 72$$

$$U(74,g,f) = 0 \qquad [9]$$

For mated females F,

$$F(t+1,g,m,f) = I(f) \times S_f(t,g,f) \times U(t,g,f) \times J(m,f) + [1 - D_f(t,g,f)] \times S_f(t,g,f) \times F(t,g,m,f) + \sum_{w \neq f}^{6} C(g,f,w) \times D_f(t,g,w) \times S_f(t,g,w) \times F(t,g,m,w) \quad \text{for } 1 \leq t \leq 72$$

$$F(74,g,m,f) = 0 \qquad [10]$$

where J is the proportion of genotype m in the total nonteneral male population, TM(f), in field f.

For males M,

$$M(t+1,g,f) = [1 - D_m(t,g,f)] \times S_m(t,g,f) \times M(t,g,f) + \sum_{w \neq f}^{6} C(g,f,w) \times D_m(t,g,w) \times S_m(t,g,w) \times M(t,g,w) \quad \text{for } 1 \leq t \leq 43$$

$$M(45,g,f) = 0 \qquad [11]$$

Oviposition. The maximum fecundity for oncemated females held under realistic, but close to optimal, nutritional conditions averages 440 viable eggs (Boetel and Fuller 1997). The model distributes the 440 over the entire oviposition period. We use the following function from the model of Onstad et al. (2001a):

$$6 \times (t - 13) \times \exp[-0.115 \times (t - 13)] \quad [12]$$

to determine the daily oviposition rate per female in age t, where $14 \leq t \leq 73$. Based on the results of Onstad et al. (2003a), we do not include a reduction in fecundity due to feeding by adults in soybean or extra vegetation.

We use $(1 - 0.01 \times t)$ to calculate the proportion of viable eggs produced by each cohort of age t during the first oviposition period (Onstad et al. 2001a). Because oviposition is random within fields, the following equations calculate the cumulative number of viable eggs in genotype k oviposited by day, d,

$$E(d + 1,k,f) = E(d,k,f) + \sum_{g=1}^{9} \sum_{m=1}^{9} \{L(k,g,m) \times \sum_{t=14}^{73} [(1 - 0.01 \times t) \times F(t,g,m,f) \times 6 \times (t - 13) \times \exp(-0.0115 \times (t - 13))]\} \quad [13]$$

for each field. $F(74,g,m,f) = 0$. Function L calculates the Mendelian proportion of eggs in genotype k resulting from the mating of genotypes g and m.

Sex Ratio and Immature Survival. We assume that the 50% of eggs are female (Onstad et al. 2001a). The probability of survival from the egg stage in the fall to the adult stage the following summer is a function of overwintering survival of the eggs, density-dependent survival of the immatures during the spring, and any additional mortality due to conventional or transgenic plant toxins. The overwintering survival of eggs, Qow, is 0.5/stage based on the range of data collected by Godfrey et al. (1995).

Density-independent toxin mortality incurred by larvae, Qtox(g,f), depends upon the dose of the toxin expressed by the roots in field f, 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 do not explicitly model the larval stages or differential responses to the toxin based on the age of larvae (Onstad et al. 2001a). 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, or 0.20 with a theoretical high, practical high, medium, or low toxin dose, respectively. With R partially recessive, survival of the heterozygotes (sr–) is 0, 0.01, 0.50, or 0.60 with a theoretical high, practical high, medium, or low toxin dose, respectively. The alleles for susceptibility to crop rotation (denoted by dashes) do not affect survival to the transgenic toxin.

We use the following equation to calculate the total number of larvae surviving overwintering and toxin mortality in each field, TL(f).

$$TL(f) = \sum_{g=1}^{9} E_{292}(g,f) \times Qow \times [1 - Qtox(g,f)] \quad [14]$$

We assume that the density-dependent survival of spring eggs to adulthood is $0.21 \times \exp(-0.058 \times EGG)$, where EGG is the density of eggs (in millions per hectare) (Onstad et al. 2003a). This function allows for a maximum of 21% larval survival. The density-dependent survival Qdd(f) for cornfields $f \geq 3$ is calculated after reducing the immature population due to overwintering and toxin mortality.

The following two equations define the density-dependent survival for larvae hatching from eggs oviposited in soybean and hatching the following year in rotated corn.

$$Qdd(4) = 0.21 \times \exp\{-.058 \times [(1 - Tr) \times TL(1)/(100 \times P_4 \times 10^6)]\}$$

$$Qdd(6) = 0.21 \times \exp\{-.058 \times [Tr \times TL(1)/(100 \times P_6 \times 10^6)]\} \quad [15]$$

The following two equations define the density-dependent survival for larvae hatching from eggs oviposited in continuouscorn:

$$Qdd(3) = 0.21 \times \exp\{-.058 \times [TL(3)/(100 \times P_3 \times 10^6)]\}$$

$$Qdd(5) = 0.21 \times \exp\{-.058 \times [TL(5)/(100 \times P_5 \times 10^6)]\} \quad [16]$$

The number of larvae per field is converted to millions per hectare by dividing by 1 million and dividing by the number of hectares in that field. The total numbers of emerging adults, TA, of genotype g in the rotated cornfields (f = 4, 6) are functions of the number of eggs laid in the soybean field the previous year:

$$TA(g,4) = (1 - Tr) \times E_{292}(g,1) \times Qow \times Qdd(4)$$

$$TA(g,6) = Tr \times E_{292}(g,1) \times Qow \times [1 - Qtox(g)] \times Qdd(6)$$

The total numbers of emerging adults in the continuous cornfields (f = 3, 5) are functions of the number of eggs laid in those fields the previous year:

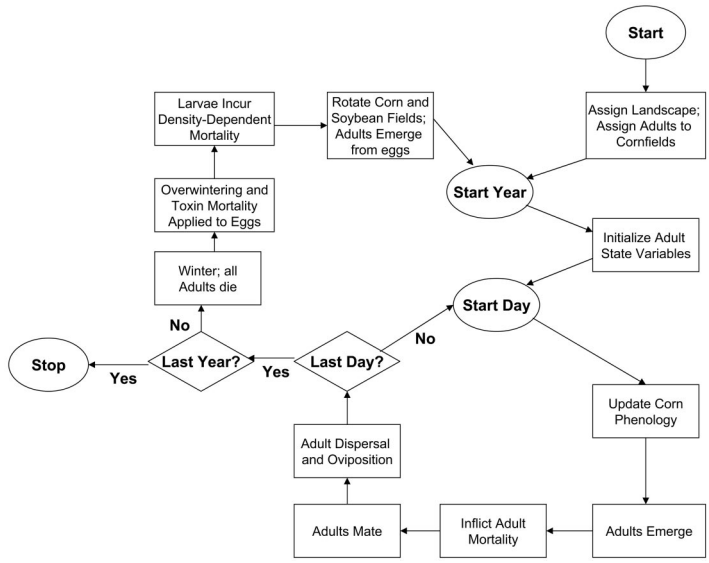

Fig. 3. Flowchart for the standard model.

$$TA(g,3) = E_{292}(g,3) \times Qow \times Qdd(3)$$

$$TA(g,5) = E_{292}(g,5) \times Qow \times [1 - Qtox(g)] \times Qdd(5)$$

For soybean and extra vegetation (f = 1, 2):

$$TA(g,f) = 0 \quad [17]$$

The numbers of female U and male M teneral adults (1-d-old) emerging each day d are

$$U_d(1,g,f) = 0.5 \times TA(g,f) \times H_1$$

$$M_d(1,g,f) = 0.5 \times TA(g,f) \times H_2 \quad [18]$$

where 0.5 is the sex ratio and H_1 and H_2 are the probability of emergence per day for females and males, respectively.

Standard Conditions. The model is programmed in FORTRAN (Intel 2003). The model has a time step of 1 d, a time horizon of 16 yr, and a spatial unit of 100 ha. The 16-yr time horizon represents the first year when the model is initialized without transgenic corn plus an additional 15-yr. The 16-yr time horizon was chosen because field observations have shown that resistance to crop rotation has evolved within 16 yr in Illinois since the first invasion by the western corn rootworm in the early 1970s (Onstad et al. 1999, 2003b). For each time step of 1 d, the rate equations are calculated before the state-variable equations. The state equations are calculated in reverse order according to age, with mated females calculated before unmated females. Each of the 16 seasons in the standard version lasts from 6 July (day 187) to 19 October (day 292) under central Illinois conditions.

A flowchart representing the processes in the standard model is shown in Fig. 3. Initial conditions for our model are the total number of adults TA(g,f) of each genotype in all cornfields at the beginning of the season. The initial number of adults is 50,000/ha corn and is distributed proportionally to the areas of continuous and rotated corn. The genotypes of the adults begin at Hardy–Weinberg equilibrium with initial R- and Y-allele frequencies of 10^{-4}. In each field besides corn, values of TA(g,f) and state variables U, M, and TU are zero at the start. In every field values for state variables E, TM, and F are zero at the start. The initial growth stage of corn, G, is 4.

Our modeled landscape is determined by the presence or absence of rotation-resistant beetles. Hereafter, we will refer to simulations with an initial Y-allele frequency >0 as simulations of areas with rotation resistance and others as simulations of areas without rotation resistance. The rotation level, R, is the sum of the proportional areas of rotated corn and soybean, which are always equal in the model ($R = Soy + C_r$). In the standard simulations of areas with rotation resistance, the landscape is defined as R = 0.85, Ex = 0.05, and $C_c = 0.10$. In the standard simulations of areas without rotation resistance, we set the initial R-allele frequency to 10^{-4} and the Y-allele frequency to 0. We studied three levels of continuous corn without rotation resistance: 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, or 1.0, the amount of land planted to C_r and Soy is 0.40, 0.20, and 0.0, respectively. In the simulations with rotation resistance, we studied several deployment strategies for transgenic crops: planting transgenic corn only in rotated cornfields (Tc = 0), planting transgenic corn only in continuous corn (Tr = 0), or planting equal proportions of continuous corn and rotated corn to a transgenic cultivar (Tc = Tr). In the simulations with no rotation resistance, we only studied the use of transgenic corn in continuous cornfields.

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 are presented as 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 allele frequencies in year 15.

Emergence Field Study. We performed a 2-yr field study to determine how western corn rootworm emergence is affected by cultivar of corn, soil insecticides, and plots infested with foxtail. In this section we first describe the experiment and then the sensitivity analysis that was performed on the model based on the results of this study.

In 2000 and 2001, we assessed the potential influence of a grass infestation (foxtail) on western corn rootworm emergence. We used a split-plot experimental design (four replications) with blocks of giant foxtail, *Setaria faberi* R.A.W. Herrm (present or absent), as the whole plots. Split plots (3 m [four rows] in width by 12.2 m in length) consisted of a transgenic rootworm-resistant hybrid expressing Cry3Bb (CRW0582Z, MON 863, 108-d hybrid), a comparable nontransgenic hybrid (CRW0589Z, isoline, 108-d hybrid), or the same nontransgenic hybrid treated with the insecticide Force (tefluthrin) (201 g [AI]/ha). The plots were planted on 12 May 2000. Within each split plot, two emergence cages (Hein et al. 1985) were deployed in late June in each of the center two rows and checked every 1.5 d for emerging beetles from 29 June until 17 August. Our cage design allowed for plant growth throughout the season, unlike Hein et al. (1985).

Grass infestations were created in the following manner. Two weeks before corn planting and the seeding of giant foxtail, a broadcast application of flumetsulam was made to control/suppress broadleaf weed species. Broadleaf weeds that emerged despite this preplant herbicide application were controlled with bentazon or dicamba, the choice dependent upon the weed species present. Giant foxtail seed was broadcast onto the appropriate whole plots with a hand-held seeder before corn planting and was incorporated shallowly. We achieved grass infestations of ≈10 plants per emergence cage. Indigenous foxtail weeds in whole plots designated as "weed free" were controlled with a preplant application of acetochlor.

In 2001, we started with the same experimental design. However, when we checked our plots for correct corn genotype, we found that all of the plants were expressing the Cry 3Bb1 protein. Therefore, we had to make several modifications to the experiment. Our whole plots still consisted of foxtail. On 25 May, 2001, we transplanted giant foxtail and green foxtail, *Setaria viridis* (L.) P. Beavu, seedlings from greenhouse flats to our plots. Our split-plot treatments consisted of the following: 1) Pioneer PN34G81 (nontransgenic seed), 2) MON 863 (treated with Force 3G), and MON 863 without Force 3G. No true isoline was used in 2001. These treatments are different from those used in 2000. Because all of the plants were expressing the Cry3Bb1 protein in the experiment plots that we established, we used adjacent border-row corn plants (PN34G81) for our "control." Ninety-six emergence cages were used (four per split plot) and were checked approximately every 2 d. Plots with identical dimensions to those used in 2000 were planted on 10 May 2001. Statistical analyses were performed with analysis of variance (ANOVA) (randomized complete block design) using SAS (SAS Institute 2002), with a significance level of 5%.

The results of this study, reported in greater detail in the results section of the manuscript, were used to perform an additional sensitivity analysis on the model. The results indicated adult beetles emerged significantly later in transgenic fields compared with nontransgenic fields. However, although beetles emerged later in transgenic fields, the cumulative proportion of beetles emerging in transgenic and nontransgenic fields approached 1.0 at approximately the same point in the season. Therefore, we believe that the delayed emergence of male and female beetles in transgenic fields is the result of high mortality caused by the transgenic crop earlier in the season (hereafter referred to as early mortality) rather than a developmental delay.

Based on the data from this study, we tested the model with early mortality for susceptible beetles emerging in transgenic fields during the first 10 or 14 d of emergence. In this analysis, we assume that 100% of the first 10 or 14 d of susceptible larval cohorts hatching in transgenic fields are killed by the transgenic crop. Therefore, with early mortality of 10 or 14 d, the first susceptible males that survive in transgenic fields emerge on day 199 or 203, respectively, and the first susceptible female beetles in transgenic fields begin emerging on day 207 or 211, respectively.

To maintain the same number of males and females emerging over these shortened periods, the overall population mortality exposed to the transgenic crop is kept constant regardless of early mortality. To simulate this, we multiplied the proportion of males or females each day in the shortened period by $(1/qe)$, where qe is the proportion of males or females that emerge in the first 10–14 d after day 189 or 197, respectively, in the standard model. In these simulations, we only tested the R recessive and R dominant cases because there are no data to suggest how the emergence of heterozygotes would be affected in the partially recessive case.

Sensitivity Analysis. In a sensitivity analysis, we studied an additional five factors. First, we studied the influence of initial allele frequency by raising the initial R- and Y-allele frequencies to 0.001 or 0.01. In areas with rotation resistance, we also tested a higher initial Y-allele frequency of 0.1 to represent areas where rotation resistance already is a severe problem. Second, we tested the effect of initial population size by raising the initial adult density to 500,000 individuals/ha and 1 million/ha. 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 $R = 0.75$ and $R = 0.95$. The landscape is $C_c = 0.15$, Soy = $C_r = 0.375$, and Ex =

0.10 if R = 0.75; or C_c = Ex = 0.025 and Soy = C_r = 0.475 if R = 0.95.

Fourth, we simulated population dynamics with two different versions of the density-dependent survival function. One was based on the model of Onstad et al. (2001a), $1/(1 + 2.42EGG^{0.7})$, which has a maximum larval survival of 100%. The other was based on the model of Crowder and Onstad (2005), $1/(2.59 + 1.29EGG^{0.88})$, 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 the field data collected by Hibbard et al. (2004). Based on their data, we simulated density-independent survival of 5% for all larvae (Crowder and Onstad 2005).

Fifth, we studied the effect of lower fecundity by susceptible adults in transgenic corn based on the data of Wilson (2003). Based on these data, we tested a lower fecundity of 50% for susceptible beetles emerging in transgenic fields (Crowder and Onstad 2005). In these simulations, we only tested the R recessive and R dominant cases for simplicity and because there is no data to suggest how the fecundity of (sr–) individuals would be affected in the partially recessive case.

For the sensitivity analysis concerning areas with rotation resistance we focused on the scenarios of planting transgenic corn only in rotated cornfields or in continuous cornfields (Tc = 0 or Tr = 0). For simulations without rotation-resistance, we performed the sensitivity analysis with all three values of continuous corn. Unless otherwise noted, in the sensitivity analysis all variables were set to standard conditions except the function being tested. In all sensitivity analyses, we simulated a refuge size of 20%.

Results

Areas with Rotation-Resistance

Simulations without Transgenic Corn. In areas with rotation-resistant phenotypes and no transgenic corn, expression of the Y-allele and the rotational level in the landscape affected the evolution of resistance to crop rotation. With Y recessive, the Y-allele frequency never exceeded 0.0001 over 15 yr with any R < 1.0.

Figure 4 shows how resistance to crop rotation evolved with Y additive or dominant and varying landscapes. With any rotational level, the Y-allele frequency increased similarly with Y additive or dominant. At the standard rotational level of 0.85, the Y-allele frequency reached ≈0.09 and 0.07 after 15 yr with Y additive and dominant, respectively. With R > 0.9, the Y-allele frequency exceeded 50% within the 15 yr time horizon with Y additive or dominant. Evolution of resistance to crop rotation occurred comparatively slowly at rotational levels ≤0.80 (Fig. 4).

Simulations with Transgenic Corn Planted to Only the Rotated Cornfield. Planting transgenic corn only in rotated cornfields was a robust strategy to prevent resistance to both transgenic corn and crop rotation in areas with rotation resistance. In all simulations with 70–95% transgenic corn planted only in rotated cornfields, neither the R- or Y-allele frequency exceeded the initial value of 0.0001 within 15 yr with any combination of refuge size, toxin dose, and gene expression.

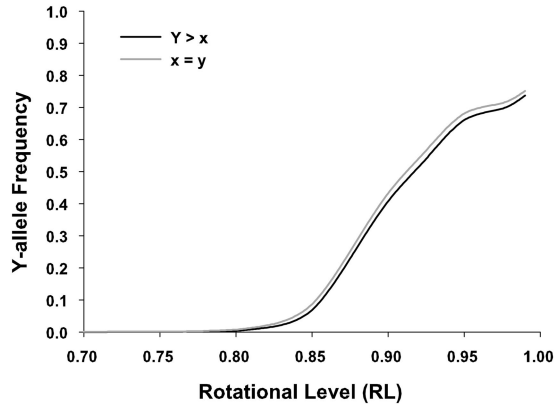

Fig. 4. Rotation-resistant (Y) allele frequency after 15-yr with varying levels of rotated landscape (R) with Y dominant (Y > x) or partially recessive (x = y). The initial Y-allele frequency is 0.0001.

Simulations with Transgenic Corn Planted to only the Continuous Cornfield. Allele expression was the most important factor affecting resistance in simulations of areas with rotation-resistant phenotypes and transgenic corn planted only in continuous cornfields. With Y recessive, the Y-allele frequency never exceeded 0.0001 within 15 yr with any combination of refuge size, toxin dose, and R-allele expression. In all cases with Y dominant, the Y-allele frequency reached 50% within 7–14 yr.

Results with Y additive were sensitive to refuge size, toxin dose, and R-allele expression (Table 1). In every case, results with a theoretical or practical high dose

Table 1. Year in which the rotation-resistant (Y) allele frequency exceeded 50% and Y-allele frequency after 15 yr in simulations of areas with rotation-resistant phenotypes and transgenic corn planted to only the continuous cornfield (Tr = 0.0), with Y additive and varying refuge sizes, toxin doses, and R-allele expression

Proportion of continuous cornfield planted to refuge[a]	Dose of toxin			
	Theoretical high	Practical high	Medium	Low
	R recessive or partially recessive			
0.05	6 (0.79)	6 (0.79)	7 (0.77)	10 (0.71)
0.10	7 (0.77)	7 (0.77)	8 (0.75)	10 (0.68)
0.20	9 (0.72)	9 (0.72)	10 (0.70)	12 (0.64)
0.30	11 (0.67)	11 (0.67)	11 (0.65)	13 (0.59)
	R dominant			
0.05	8 (0.66)	8 (0.66)	10 (0.61)	12 (0.59)
0.10	8 (0.67)	8 (0.67)	10 (0.62)	12 (0.59)
0.20	10 (0.67)	10 (0.67)	11 (0.63)	13 (0.59)
0.30	11 (0.65)	11 (0.65)	12 (0.62)	14 (0.56)

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

Table 2. Year in which the transgenic corn resistant (R) allele frequency exceeded 50% and R-allele frequency in year 15 in simulations of areas with rotation-resistant phenotypes and transgenic corn planted to only the continuous cornfield (Tr = 0.0), with R partially recessive and varying refuge sizes, toxin doses, and Y-allele expression

Proportion of continuous cornfield planted to refuge[a]	Dose of toxin			
	Theoretical High	Practical High	Medium	Low
	Y recessive			
0.05	>15 (0.0001)	>15 (0.0004)	7 (0.99)	11 (0.93)
0.10	>15 (0.0001)	>15 (0.0002)	8 (0.99)	12 (0.84)
0.20	>15 (0.0001)	>15 (0.0001)	12 (0.88)	>15 (0.41)
0.30	>15 (0.0001)	>15 (0.0001)	>15 (0.27)	>15 (0.08)
	Y additive or dominant			
0.05	>15 (0.0001)	>15 (0.0001)	>15 (0.04)	>15 (0.02)
0.10	>15 (0.0001)	>15 (0.0001)	>15 (0.02)	>15 (0.02)
0.20	>15 (0.0001)	>15 (0.0001)	>15 (0.01)	>15 (0.01)
0.30	>15 (0.0001)	>15 (0.0001)	>15 (0.005)	>15 (0.004)

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

were the same. With these doses, resistance to crop rotation evolved more rapidly compared with either a medium or low dose. Resistance also developed more rapidly with R dominant compared with R recessive or partially recessive (Table 1).

With R recessive, the R-allele frequency never exceeded 0.0001 within 15 yr with any combination of refuge size, toxin dose, and Y-allele expression. The results with R partially recessive were sensitive to refuge size, toxin dose, and Y-allele expression (Table 2). In all cases with a theoretical or practical high toxin dose, the R-allele frequency never exceeded 0.0004 within the 15-yr simulation period (Table 2). With a medium or low dose and Y recessive, there were several cases where the R-allele frequency reached 50% within 15 yr. However, with R partially recessive and Y additive or dominant, the R-allele frequency never reached 50% for any combination of toxin dose and refuge size, reaching a maximum value of 0.04 after 15 yr (Table 2).

With R dominant, results were sensitive to refuge size, toxin dose, and Y-allele expression (Table 3). With R dominant and Y recessive, the R-allele frequency exceeded 50% within 15 yr in every case, with the R-allele frequency reaching 0.63–0.92 after 15 yr. With R dominant and Y additive, with 5% refuge there were cases where the R-allele frequency exceeded 50% within 15 yr, and the R-allele frequency reached at least 0.11 after 15 yr with any refuge size and toxin dose. With R and Y dominant, the R-allele frequency never exceeded 50% within the 15-yr simulation period, reaching 0.06–0.36 after 15 yr (Table 3).

Simulations with Transgenic Corn Planted to both Cornfields. Planting an equal proportion of the landscape to both continuous and rotated cornfields was a robust strategy to prevent resistance to crop rotation. With $0.70 \leq Tc = Tr \leq 0.95$, the Y-allele frequency never reached 50% within the 15-yr simulation period with any combination of refuge size, toxin dose, and allele expression for either trait. With Y additive or dominant, the Y-allele frequency after 15 yr reached 0.05–0.11 with any dose and refuge size.

Table 3. Year in which the transgenic corn resistant (R) allele frequency exceeded 50% and R-allele frequency in year 15 in simulations of areas with rotation-resistant phenotypes and transgenic corn planted to only the continuous cornfield (Tr = 0.0), with R dominant and varying refuge sizes, toxin doses, and Y-allele expression

Proportion of continuous cornfield planted to refuge[a]	Dose of toxin			
	Theoretical high	Practical high	Medium	Low
	Y recessive			
0.05	4 (0.92)	4 (0.92)	5 (0.91)	8 (0.86)
0.10	6 (0.91)	6 (0.91)	6 (0.90)	9 (0.84)
0.20	8 (0.87)	8 (0.87)	8 (0.86)	11 (0.77)
0.30	11 (0.79)	11 (0.79)	11 (0.77)	14 (0.63)
	Y additive			
0.05	13 (0.57)	13 (0.58)	11 (0.64)	>15 (0.49)
0.10	>15 (0.46)	>15 (0.47)	14 (0.55)	>15 (0.41)
0.20	>15 (0.26)	>15 (0.26)	>15 (0.35)	>15 (0.24)
0.30	>15 (0.12)	>15 (0.12)	>15 (0.16)	>15 (0.11)
	Y dominant			
0.05	>15 (0.36)	>15 (0.38)	>15 (0.46)	>15 (0.30)
0.10	>15 (0.25)	>15 (0.26)	>15 (0.35)	>15 (0.23)
0.20	>15 (0.12)	>15 (0.12)	>15 (0.18)	>15 (0.13)
0.30	>15 (0.05)	>15 (0.05)	>15 (0.08)	>15 (0.06)

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

With R recessive the R-allele frequency never exceeded 0.0001 within 15 yr with any combination of refuge size, toxin dose, and Y-allele expression. With R partially recessive, results were sensitive to refuge size and toxin dose in the corn plant but not Y-allele expression (Table 4). With R partially recessive, the R-allele frequency never exceeded 0.0004 within 15 yr with a theoretical or practical high toxin dose. A me-

Table 4. Year in which the transgenic corn resistant (R) allele frequency exceeded 50% and R-allele frequency in year 15 in simulations of areas with rotation-resistant phenotypes and transgenic corn planted to both continuous and rotated cornfields (Tc = Tr), with R partially recessive or dominant, and varying refuge sizes. Results were the same with Y recessive, partially recessive, or dominant

Proportion of both cornfields planted to refuge[a]	Dose of toxin			
	Theoretical high	Practical high	Medium	Low
	R partially recessive			
0.05	>15 (0.0001)	>15 (0.0004)	7 (0.99)	11 (0.93)
0.10	>15 (0.0001)	>15 (0.0002)	8 (0.99)	12 (0.84)
0.20	>15 (0.0001)	>15 (0.0001)	12 (0.88)	>15 (0.41)
0.30	>15 (0.0001)	>15 (0.0001)	>15 (0.27)	>15 (0.08)
	R dominant			
0.05	4 (0.92)	4 (0.92)	5 (0.91)	8 (0.86)
0.10	6 (0.91)	6 (0.91)	6 (0.90)	9 (0.84)
0.20	8 (0.87)	8 (0.87)	8 (0.86)	11 (0.77)
0.30	11 (0.79)	11 (0.79)	11 (0.77)	14 (0.63)

[a] Proportion of cornfields planted to transgenic cultivar = 1 − proportion refuge.

Table 5. Year in which the transgenic corn resistant (R) allele frequency exceeded 50% and R-allele frequency in year 15 in simulations of areas without rotation-resistant phenotypes, R dominant, 20% continuous corn, and varying refuge sizes and toxin doses in only the continuous cornfield (Tr = 0.0)

Proportion of continuous cornfield planted to refuge[a]	Dose of toxin			
	Theoretical high	Practical high	Medium	Low
0.05	4 (0.92)	4 (0.92)	5 (0.91)	8 (0.86)
0.10	6 (0.91)	6 (0.91)	6 (0.90)	9 (0.84)
0.20	8 (0.87)	8 (0.87)	9 (0.85)	11 (0.77)
0.30	11 (0.79)	11 (0.79)	11 (0.77)	14 (0.62)

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

dium dose of toxin was effective at preventing resistance only with 30% refuge planted to each cornfield, although the R-allele frequency reached 0.27 after 15 yr (Table 4). With a low dose, resistance to transgenic corn did not evolve within 15 yr only with 20% or more of both fields planted to refuge, although the R-allele frequency reached at least 0.08 in every case after 15 yr (Table 4).

Similarly with R dominant, the results were sensitive to refuge size and toxin dose but were unaffected by the expression of the Y-allele (Table 4). With any refuge size or toxin dose and R dominant, the R-allele frequency exceeded 50% within 15 yr, with the R-allele frequency reaching (0.63–0.92). Increasing the refuge size and decreasing the toxin dose slowed the evolution of resistance to transgenic corn (Table 4).

Areas without Rotation-Resistance

Expression of the R-allele was the most important factor affecting the evolution of resistance in simulations without rotation-resistance. In areas with 20% continuous corn, the R-allele frequency never exceeded 0.0001 within 15 yr with R recessive and any combination of refuge size and toxin dose. With R partially recessive, the R-allele frequency never exceeded 0.0004 within 15 yr with a theoretical or practical high toxin dose. With a medium dose and R partially recessive, only with 30% refuge did the R-allele frequency not exceed 50% within 15 yr, reaching 0.25. With a low dose and R partially recessive, the R-allele frequency did not exceed 50% only with 20 and 30% refuge in the continuous corn, reaching 0.38 and 0.07, respectively. These results are similar to the results with Tc = Tr in areas with rotation resistance.

With R dominant, toxin dose in the corn plant and refuge size in the continuous corn both affected the evolution of the R-allele (Table 5). With any toxin dose and refuge size, the R-allele frequency exceeded 50% within 15 yr, reaching 0.62–0.92. In every case increasing the refuge size delayed the evolution of resistance. In all cases, the results did not differ by >1 yr with a theoretical high, practical high, or medium dose of toxin, whereas a low dose of toxin increased the time for the R-allele frequency to reach 50% by 3–4 yr compared with the higher doses (Table 5). The results presented in Table 5 are similar to the results shown in Table 4, indicating that evolution of the R-allele occurs similarly between areas without rotation resistance and transgenic corn only in the continuous cornfields and areas with rotation resistance and transgenic corn planted in both cornfields.

The results were similar in simulations of areas with 60 or 100% continuous corn and no rotation resistance. With R recessive, the R-allele frequency never exceeded 50% within 15 yr in simulations of areas with 60 or 100% continuous corn. With R partially recessive, the results with 60 or 100% continuous corn never differed by >1 yr from the results with 20% continuous corn.

Emergence Study

The results of the emergence study indicated that total emergence of both male and female western corn rootworm beetles is significantly affected by the cultivar of corn but not by insecticides or foxtail infestations. The results of male emergence are presented first, followed by results of female emergence, and finally results of combined male and female emergence are presented.

The influence of the giant foxtail infestation on total emergence of western corn rootworm males was not significant ($F_{1,3} = 0.94$; $P = 0.40$). The effect of cultivar on total emergence of western corn rootworm males was significant ($F_{2,84} = 12.92$; $P = 0.0001$). No significant interaction between giant foxtail infestation and hybrid treatments was evident ($F_{2,84} = 1.65$; $P = 0.20$). Therefore, total emergence data were pooled across the whole-plot treatments (giant foxtail). Pooled means for total emergence (males per cage) for the isoline, MON 863, and the isoline + tefluthrin were 8.2, 3.1, and 1.4, respectively. The least significant difference (LSD) for these means ($\alpha = 0.05$) was 2.8. Thus, significantly more western corn rootworm males emerged in the isoline treatment. No statistical difference in total emergence of western corn rootworm males was detected between MON 863 and the isoline treated with tefluthrin at planting.

The results of the field study on male emergence are shown in Fig. 5. In 2000 and 2001, we captured 168 and 69 males, respectively, in control plots and 51 and three males, respectively, in transgenic plots. For both years, the first males in control plots emerged approximately Julian day 180, and 50% of males had emerged by Julian day 200 (Fig. 5a, b). All males captured in control plots had emerged by Julian day 228 in 2000. In contrast, in 2000 in transgenic fields we observed the first males, 50% emergence, and the last male on Julian days 191, 202, and 226, respectively. The emergence of males in transgenic plots was delayed, compared with the emergence of males in control plots, especially during the early period of emergence (Fig. 5). In 2000, the emergence curve for males in control plots was similar to the emergence curve for males in plots treated with a soil insecticide (Fig. 5a). In 2001, when fewer beetles were observed in treated plots, the emergence curve for males in transgenic plots was

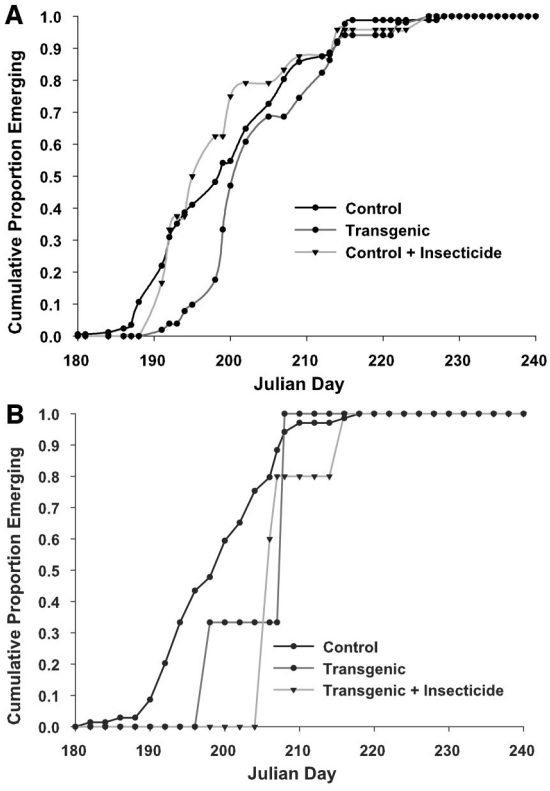

Fig. 5. Cumulative emergence of male western corn rootworm for (a) 2000 and (b) 2001 for control, transgenic and control + soil insecticide or transgenic + soil insecticide treated plots.

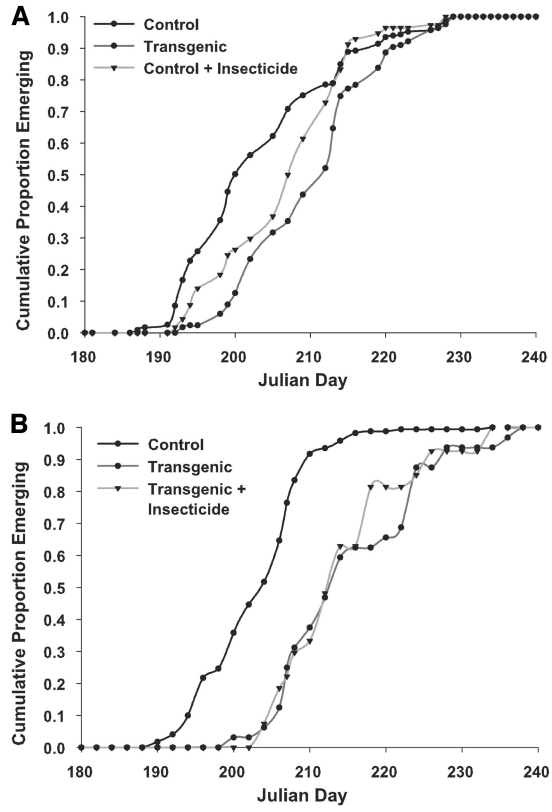

Fig. 6. Cumulative emergence of female western corn rootworm for (a) 2000 and (b) 2001 for control, transgenic and control + soil insecticide or transgenic + soil insecticide treated plots.

similar to the emergence curve for males in transgenic plots treated with a soil insecticide (Fig. 5b).

The effect of the giant foxtail infestation on total emergence western corn rootworm females was not significant ($F_{1,3} = 2.13$; $P = 0.24$). Emergence of females was affected significantly by cultivar selection ($F_{2,84} = 6.96$; $P = 0.002$). The interaction of giant foxtail with cultivars relative to total emergence of western corn rootworm females was not significant ($F_{2,84} = 0.29$; $P = 0.75$). Because the interaction of whole-plot and split-plot treatments was not significant, emergence data were pooled across giant foxtail infestations. Pooled means for total emergence (females per cage) for the isoline, MON 863, and the isoline + tefluthrin were 13.9, 8.3, and 6.3, respectively. The LSD for these means ($\alpha = 0.05$) was 4.2. Thus, significantly fewer female western corn rootworms emerged from the MON 863 and isoline + tefluthrin treatments than from the isoline alone.

The results of the field study on female emergence are shown in Fig. 6. In 2000 and 2001, we captured 233 and 170 females, respectively, in control plots and 167 and 32 females, respectively, in transgenic plots. For 2000 and 2001, the first females captured in control plots occurred on Julian days 187 and 190, respectively, with 50% emergence by Julian day 200 and 204, respectively. All females in control plots were captured by Julian day 229 and 234 in 2000 and 2001, respectively. For 2000 and 2001, the first females captured in transgenic plots occurred on Julian days 193 and 198, respectively, with 50% emergence occurring by Julian day 212. All females in transgenic plots were captured by Julian day 229 and 238 in 2000 and 2001, respectively. Similar to male emergence, females emerged from transgenic plots later than females from control plots (Fig. 6a, b), especially at beginning of emergence. In 2001, the emergence curve for females in transgenic plots was similar to the emergence curve of females in transgenic plots treated with a soil insecticide (Fig. 6b).

The effect of giant foxtail on total (combined male and female data) emergence of western corn rootworms was not significant ($F_{1,3} = 5.51$; $P = 0.10$). The influence of cultivar on total emergence of western corn rootworms was highly significant ($F_{2,84} = 11.58$; $P = 0.0001$). No significant interaction was noted between the whole-plot and split-plot treatments with respect to total emergence (males and females combined) of western corn rootworms ($F_{2,84} = 0.29$; $P = 0.75$).

Figure 7 shows the influence of early mortality during the first 10 d of emergence on the simulated densities of adult beetles over the course of one season

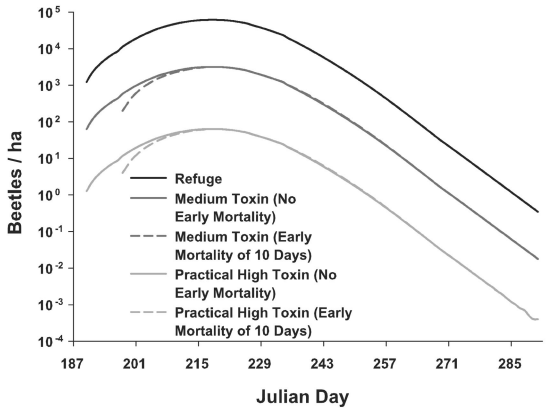

Fig. 7. Simulated adult densities (beetles per hectare) during the second season (the first with transgenic corn), for beetles emerging in refuge or transgenic fields with two doses of toxin. The emergence of beetles in transgenic fields was simulated with and without early mortality of 10 d. Note that densities before first positive values in figure are zero.

with two toxin doses. The densities shown are for the second year of the simulation (the first year is simulated without transgenic corn). With both the practical high or medium dose, early mortality results in delayed emergence during the early part of the season and decreased adult densities before approximately Julian date 215. Figure 7 shows that the dose of the toxin and the type of field (refuge or transgenic) has a much greater effect on adult densities compared with early mortality.

Sensitivity Analysis

Areas with Rotation Resistance. *Simulations with Transgenic Corn Planted to only the Rotated Cornfield.* The model was not sensitive to any of the six factors studied in the sensitivity analysis in simulations of areas with rotation-resistant phenotypes, Tc = 0 and Tr = 0.80. None of the six factors tested resulted in the R- or Y-allele frequency exceeding 0.0001 within the 15-yr simulation period. Planting transgenic corn only in rotated cornfields resulted in a decrease in the Y-allele frequency over time even in simulations with an initial Y-allele frequency of 0.1. Results of the model indicate that planting transgenic corn only in rotated cornfields was an effective strategy to reverse the evolution of the Y-allele even at initial Y-allele frequencies >0.9.

Simulations with Transgenic Corn Planted to only the Continuous Cornfield. In simulations of areas with rotation resistance, Tc = 0.80 and Tr = 0, increasing the initial population size to 500,000 or 1 million individuals never changed the time for the R- or Y-allele frequency to reach 50% by >1 yr. Imposing a fecundity cost for susceptible adults emerging in transgenic cornfields did not affect the time for the R-allele frequency to reach 50%. However, fecundity costs did affect the evolution of the Y-allele in some cases. The greatest change occurred with a low dose, Y additive or dominant, and R recessive, where the time for the Y-allele frequency to reach 50% was shortened from 12 to 9 yr, a 25% reduction in time. Neither of these factors resulted in the final allele frequencies differing by >0.01 compared with the standard.

Table 6. Year in which the rotation-resistant (Y) allele frequency exceeded 50% and Y-allele frequency in year 15 in simulations of areas with rotation-resistant phenotypes and transgenic corn planted to only the continuous cornfield (Tr = 0.0), with varying rotational levels in the landscape, toxin doses, and Y- and R-allele expression

Proportion of rotated landscape	Dose of toxin			
	Theoretical high	Practical high	Medium	Low
	Y additive, R recessive or partially recessive			
0.95	6 (0.79)	6 (0.79)	6 (0.79)	7 (0.77)
0.85 (standard)	9 (0.72)	9 (0.72)	10 (0.70)	12 (0.64)
0.75	12 (0.65)	12 (0.65)	13 (0.61)	>15 (0.45)
	Y additive, R dominant			
0.95	6 (0.79)	6 (0.79)	6 (0.79)	7 (0.77)
0.85 (standard)	10 (0.67)	10 (0.67)	11 (0.63)	13 (0.59)
0.75	>15 (0.31)	>15 (0.31)	>15 (0.22)	>15 (0.14)
	Y dominant, R recessive or partially recessive			
0.95	7 (0.74)	7 (0.74)	7 (0.74)	7 (0.72)
0.85 (standard)	9 (0.69)	9 (0.69)	10 (0.68)	12 (0.62)
0.75	11 (0.64)	11 (0.64)	13 (0.61)	15 (0.46)
	Y dominant, R dominant			
0.95	7 (0.74)	7 (0.74)	7 (0.73)	7 (0.72)
0.85 (standard)	9 (0.67)	9 (0.67)	10 (0.64)	12 (0.59)
0.75	>15 (0.36)	>15 (0.35)	>15 (0.26)	>15 (0.17)

The time for the R- or Y-allele frequency to reach 50% was shortened by up to 2 yr with the density-dependent function that allowed 39 or 100% maximum larval survival compared with the standard simulations. With the density-independent function for larval survival, the time for the R- or Y-allele frequency to reach 50% increased by up to 1 yr compared with the standard simulations. In no case did the final R- or Y-allele frequency after 15 yr differ by >8% compared with the standard.

Imposing early mortality for susceptible beetles emerging the first 10 or 14 d in transgenic fields shortened the time for the R- and Y-allele frequency to reach 50% by up to 2 yr compared with the standard simulations. The largest change occurred with a low toxin dose, Y recessive and R dominant, where the time for the Y-allele to reach 50% with early mortality was shortened from 12 to 10 yr, a 16.7% reduction in time. With early mortality, the final R- or Y-allele frequencies were always within 5% of the standard simulations.

Changing the level of rotation in the landscape to 75 or 95% did not affect the time for the Y-allele to exceed 50% with Y recessive. Table 6 compares the evolution of the Y-allele with R = 0.75 or 0.95 to the standard (R = 0.85) with Y additive or dominant. In every case, increasing the rotational level decreased the number of years for the Y-allele frequency to reach 50% and increased the Y-allele frequency in year 15. In contrast, decreasing the rotational level to R = 0.75 increased the number of years for the Y-allele frequency

to reach 50% and decreased the Y-allele frequency in year 15 (Table 6). The greatest percentage increase or decrease in the Y-allele frequency after 15 yr caused by changing the rotational level was 31 or 76%, respectively. Changing the rotational level in the landscape affected the time for the R-allele frequency to reach 50% only in the following case. With R dominant and Y additive or recessive and $R = 0.75$, the time for the R-allele frequency to reach 50% was shortened to 10–12 yr with any toxin dose, compared with the standard simulations where resistance to transgenic corn did not evolve.

With an initial R-allele frequency of 0.001 or 0.01 and R recessive, the R-allele frequency never exceeded 0.001 or 0.02, respectively, after 15 yr in any case. In contrast, with R dominant and an initial R-allele frequency of 0.001 or 0.01, the R-allele frequency reached 50% within 15 yr in every case. With R partially recessive, resistance to transgenic corn did not evolve with a theoretical or practical high dose with an increased initial R-allele frequency. Raising the R-allele frequency to 0.001 or 0.01 did result in evolution of resistance to transgenic corn with a medium or low dose in several cases with R partially recessive. This differed from the standard simulations where resistance did not occur within 15 yr except in one case with a medium toxin dose. The greatest percentage change occurred with a medium dose, Y recessive, and an initial R-allele frequency of 0.01, where the R-allele frequency exceeded 50% in year 6, a 50% reduction in time compared with the standard simulations.

Increasing the initial R-allele frequency did not affect the time for the Y-allele frequency to reach 50% or the Y-allele frequency in year 15 in most cases. However, in some simulations, raising the initial R-allele frequency resulted in an increase in the number of years for the Y-allele frequency to reach 50%. In these cases the number of years for the Y-allele frequency to reach 50% increased by as much as 20–30% with an increased initial R-allele frequency compared with the standard simulations.

Increasing the initial Y-allele frequency to 0.001 or 0.01 did not affect the evolution of resistance to crop rotation with Y recessive. With an initial Y-allele frequency of 0.001 and 0.01, the number of years for the Y-allele frequency to reach 50% was reduced by 11–23 and 30–40%, respectively, with Y additive or dominant compared with the standard simulations. Increasing the Y-allele frequency to these levels did not affect resistance to transgenic corn in any case. Increasing the initial Y-allele frequency to 0.1 shortened the time for the Y-allele frequency to reach 50% to 5–7 yr with any combination of toxin dose and allele expression for both traits. However, with this increased value, the R-allele frequency never reached 50% in any case.

Areas without Rotation Resistance. Increasing the initial population size to 500,000 or one million individuals/ha did not affect the time for the R-allele frequency to reach 50% or the R-allele frequency in year 15 with any combination of toxin dose, gene expression, or proportion landscape planted to continuous corn. Similarly, imposing a fitness cost for susceptible beetles emerging in transgenic corn of 25 or 50% did not change the time for the R-allele frequency to reach 50% by >1 yr or change the final R-allele frequency in year 15 by >0.01.

With any density dependence function, the time for the R-allele frequency to reach 50% was not affected with R recessive. Similarly, the results with the density-independent function that allowed 5% survival were the same as results with the standard density-dependence function in every case. With R partially recessive or dominant, using a function that allowed a maximum survival of 39% shortened times by up to 1 yr compared with the standard. With the density-dependent function that allowed 100% survival, the time for the R-allele frequency to reach 50% was shortened by up to 4 yr.

The greatest change occurred with a medium dose and 100% continuous corn, where the R-allele frequency reached 50% within 6 yr, representing a 33% reduction in the number of years for the R-allele frequency to reach 50% compared with the standard.

Early mortality for susceptible beetles emerging the first 10 or 14 d in transgenic fields shortened the time for the R-allele frequency to reach 50% by 1 yr with a medium or low dose of toxin but did not affect the time for the R-allele frequency to reach 50% with a theoretical or practical high toxin dose. Early mortality did not affect the R-allele frequency in year 15 by >3% in any case.

Increasing the R-allele frequency to 0.001 or 0.01 affected the time for the R-allele frequency to reach 50% with R partially recessive or dominant in several cases. With R dominant, the time for the R-allele frequency to reach 50% was shortened to 6–9 or 4–6 yr with an initial allele frequency of 0.001 or 0.01, respectively, and any toxin dose, compared with 8–11 yr under standard conditions. With R partially recessive, the time for the R-allele frequency to reach 50% was decreased with a medium or low toxin dose but not affected with the higher doses.

Discussion

Results of the model indicate that transgenic corn, when planted only in rotated cornfields, is a robust strategy to prevent resistance to both crop rotation and transgenic corn in areas with rotation-resistant phenotypes. This strategy is effective at preventing resistance to crop rotation because it places tremendous selection pressure on rotation-resistant individuals emerging in first-year cornfields. In addition, the continuous cornfield serves as an additional refuge to prevent resistance to transgenic corn.

Even when the model was simulated with initial Y-allele frequencies >0.9, planting transgenic corn only in rotated cornfields caused a decrease in the Y-allele frequency over time. These results differ from two other models that used a generational time step to simulate the evolution of resistance to crop rotation (Onstad et al. 2003a, Crowder and Onstad 2005). In these models, resistance to crop rotation cannot be

reversed by planting transgenic corn to rotated cornfields if the initial Y-allele frequency >0.01. Further investigation of this finding may be warranted.

In areas where rotation-resistance may be a future problem, planting transgenic corn only in continuous cornfields is not an effective strategy to prevent resistance to either trait. With Y additive or dominant, planting transgenic corn only in continuous cornfields resulted in faster evolution of the Y-allele than in simulations with no transgenic corn in the landscape. In general, the R-allele expression did not affect the evolution of resistance to crop rotation, but the Y-allele expression did affect the evolution of resistance to transgenic corn. Resistance to transgenic corn develops fastest when Y is recessive. With Y recessive, fewer adult beetles will travel outside of corn, resulting in more beetles remaining within the continuous cornfield under greater selection pressure.

In areas without rotation resistance, the amount of landscape planted to continuous corn did not affect the evolution of resistance to transgenic corn. Results were similar in landscapes with 20, 60, or 100% continuous corn. 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 15 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).

Farmers considering the use of transgenic corn to control western corn rootworm should determine whether they are located in an area with the risk of invasion by rotation-resistant phenotypes. If farmers are located in an area where rotation-resistant western corn rootworms already pose a problem or may cause problems in the near future, they should consider a management approach of planting transgenic corn only in first-year cornfields. Results of the model also indicate that, in these areas, planting an equal proportion of both the continuous and rotated cornfields to a transgenic cultivar (Tc = Tr) is an effective strategy to prevent rotation resistance from developing. However, farmers planting transgenic corn to both continuous and rotated cornfields must carefully manage their fields to prevent evolution of resistance to the transgenic crop.

Refuge size and toxin dose also are important management considerations. In areas with rotation-resistant phenotypes, planting more landscape to refuge fields should slow the resistance to transgenic corn but may actually increase the rate of evolution of resistance to crop rotation. However, smaller refuges increase the rate of evolution of resistance to transgenic corn but slow the development of resistance to crop rotation. In general, the high and low doses of transgenic corn were more effective at delaying resistance to transgenic corn than a medium 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. However, the theoretical and practical high doses were not as effective at preventing resistance to crop rotation as the medium or low doses. Based on these factors, managers must carefully balance the risks presented to them from both rotation-resistant and transgenic-resistant populations of western corn rootworms when devising the best management strategy for their area.

Results of the sensitivity analysis indicate that initial allele frequency, rotational level in the landscape, and density dependence are the three most important factors affecting the evolution of resistance to both traits. In general, increasing the initial population size and imposing fecundity costs or early mortality for susceptible beetles emerging in transgenic corn did not significantly affect the results, although resistance did develop slightly faster than under standard conditions.

Results of the emergence study indicate differences in emergence of male and female western corn rootworm adults based on cultivar of corn. The results showed that adults emerged later in the season from transgenic compared with nontransgenic plots. Soil insecticide treatments and foxtail infestations did not significantly affect the emergence of males or females. Incorporating early mortality in the model increases the proportion of resistant individuals in transgenic fields for the first 10–14 d of emergence, which increases the mating frequency between resistant individuals. By incorporating early mortality into the model we could analyze how significant this change in mating is on evolution. Results of the model indicated that imposing early mortality for susceptible beetles emerging in transgenic corn did not significantly affect the evolution of resistance to either trait.

Managers must make decisions about the use of transgenic corn despite several uncertainties. Because managers are unlikely to know the initial resistant allele frequencies, the expression of these alleles, and the survival of different genotypes feeding on transgenic corn roots, they should focus on implementing strategies that have the best chance of success over a broad range of possibilities. The western corn rootworm is a species with a long history of developing resistance to control measures, such as chemical insecticides and crop rotation (Ball and Weekman 1962, 1963; Meinke et al. 1998; Metcalf 1983; Miota et al. 1998; O'Neal et al. 1999; Scharf et al. 1999). Given this background, transgenic corn must be managed cautiously to prevent resistance to this technology.

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 the resistant traits 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) investigated a row-strip configuration for planting transgenic corn and found that this configuration was not as effective at delaying resistance as a block configuration. However, with our new functions for dispersal that allow beetles to move into different fields based on their proportional area within the region, we believe that the results would be similar regardless of the refuge configuration. Fourth, we did not vary the location of the refuge in continuous corn from year to year. Changes in these assumptions could produce other outcomes.

Results of this model will help provide insight on future research that needs to be conducted. A laboratory bioassay and standard crossing of individuals could be performed to help determine the gene expression for both resistance traits. A future model should consider the use of transgenic corn in more complex refuge configurations or seed mixtures. The economic costs and benefits of using transgenic corn also should be studied more extensively. Another future model should consider the use of insecticides in conjunction with transgenic corn. Further field studies should be conducted to determine the true impact of density-dependent survival.

We believe that our results show that transgenic corn may potentially be an effective management approach for rotation-resistant populations of western corn rootworm in the future. Although management recommendations and decisions by growers must be made in the face of many uncertainties, transgenic corn has the potential to improve yields significantly in areas with severe rootworm problems. However, until we have more knowledge about specific aspects of this particular system, growers must carefully weigh the costs and benefits of different management strategies to fit their region and needs.

Acknowledgments

We thank Tony Shelton and two reviewers for comments that greatly improved the manuscript. We also thank Chip Guse for providing advice and assistance with the computer code and the University of Illinois and the College of ACES for supporting this research with a Jonathan Baldwin Turner Fellowship. This work was supported by a grant to Onstad from the USDA Biotechnology Risk Assessment program. The ideas expressed in this paper may not represent those of the USDA.

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Received 12 May 2004; accepted 2 November 2004.

Appendix 1

d day of the year;

f index for field;

g index for genotype;

h index for nontransgenic (1) and transgenic (2) fields;

i secondary index for field;

j tertiary index for field;

k secondary index for genotype of eggs;

m index for genotype of mate (male);

p proportion of males remaining in natal field;

qe proportion of males or females emerging in first 10 or 14 d (used in sensitivity analysis);

r allele for resistance to transgenic corn;

s allele for susceptibility to transgenic corn;

t age of adult in days;

x allele for normal movement within corn;

y allele for random movement throughout landscape;

C proportion of dispersers entering field f from field i each day;

C_c proportion of land planted to continuous corn;

C_r proportion of land planted to rotated corn;

D proportion of adults leaving field f per day;

E number of eggs;

Ex proportion of land planted to extra (noncorn) vegetation;

F number of mated female adults;

G growth stage of corn;

I probability of an unmated female being mated per day;

J proportion of males in genotype;

L Mendelian proportions of eggs resulting from mating of two genotypes;

M number of male adults;

P proportion of region in field f;

Q probability of survival for immatures, proportion/stage;

R allele for resistance to transgenic corn

S probability of survival for adults per day or allele for susceptibility to transgenic corn;

Soy proportion of land planted to soybean;

T proportion of corn crop that is transgenic;

U number of unmated females;

X allele for normal movement within corn;

Y allele for random movement throughout landscape;

EGG millions of eggs per ha

TA total number of adults emerging in field;

TL total number of larvae for calculation of Qdd;

TM total number of nonteneral males;

TU total number of unmated females.