Research Article

Modeling a western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), maturation delay and resistance evolution in *Bt* corn

Jung koo Kang,[a,*] Christian H Krupke,[b] Alexzandra F Murphy,[c] Joseph L Spencer,[d] Michael E Gray[e] and David W Onstad[f]

Abstract

BACKGROUND: Emergence delay and female-skewed sex ratios among adults of *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) from *Bt* corn have been reported in field studies. The authors used a simulation model to study the effect of a maturation delay and a female-skewed sex ratio for *D. v. virgifera* emerging from *Bt* corn on the evolution of *Bt* resistance.

RESULTS: The effect of skewed toxin mortality in one sex on evolution of *Bt* resistance was insignificant. An emergence delay among resistant beetles from *Bt* corn slowed resistance evolution. A shift in the time of emergence for homozygous susceptible beetles from *Bt* corn did not have a significant effect on the evolution of *Bt* resistance in *D. v. virgifera*.

CONCLUSION: This simulation study suggested that skewed toxin mortality in one sex and an emergence delay for beetles in *Bt* corn are not major concerns for managing resistance by *D. v. virgifera* to single-toxin or pyramided *Bt* corn.
ⓒ 2013 Society of Chemical Industry

Supporting information may be found in the online version of this article.

Keywords: *Diabrotica virgifera virgifera*; resistance management; *Bt* corn

1 INTRODUCTION

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is the most serious insect pest of corn in the midwestern United States.[1] *D. v. virgifera* is univoltine. The eggs overwinter in soil and begin to hatch in late May and early June.[1] Larvae feed on corn roots for 3–4 weeks. Their feeding reduces water uptake and nutrient absorption, facilitates the entry of stalk- and root-infesting microorganisms and makes plants susceptible to lodging, which leads to yield loss. *D. v. virgifera* has a long history of developing resistance to a variety of insecticides and has even developed behavioral resistance to crop rotation.[1–5] Transgenic corn hybrids expressing coleopteran-specific *Bacillus thuringiensis* toxins have been used to manage *D. v. virgifera* since 2003.[6] The first field-evolved *Bt* resistance in *D. v. virgifera* was discovered in Iowa in 2009.[7]

Understanding pest behavior is critical to insect resistance management (IRM) and integrated pest management (IPM), but quantification of adult behavior is challenging.[8,9] The likelihood of mating between beetles emerging from *Bt* corn targeting *D. v. virgifera* and those from non-*Bt* corn is affected by spatial and temporal separation. The spatial dynamics of male and female beetle emergence from *Bt* and non-*Bt* corn on the evolution of *Bt* resistance has been investigated in several studies.[10,11] Field studies reported partial temporal separation between beetles emerging from *Bt* and non-*Bt* corn.[11–13] They raised concerns regarding management of *Bt* resistance in *D. v. virgifera* because the temporal shift decreases the frequency of mating between beetles emerging from *Bt* and non-*Bt* corn.

Field and greenhouse studies showed that the sex ratios of beetles emerging in corn expressing Cry3Bb1 or Cry34Ab1/Cry35Ab1 are usually more female skewed than those for beetles emerging in non-*Bt* corn.[12–17] If *Bt* toxins kill one sex

---

*　Correspondence to: Jung koo Kang, Department of Entomology, University of Illinois, 320 Morrill Hall, 505 South Goodwin Ave., Urbana, IL 61801, USA. E-mail: jungkoo.kang@gmail.com*

a　*Department of Entomology, University of Illinois, Urbana, IL, USA*

b　*Department of Entomology, Purdue University, West Lafayette, IN, USA*

c　*Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston, OR, USA*

d　*Illinois Natural History Survey, Forbes Natural History Building, University of Illinois, Champaign, IL, USA*

e　*Department of Crop Sciences, University of Illinois, Urbana, IL, USA*

f　*DuPont Agricultural Biotechnology, Experimental Station, Wilmington, DE, USA*
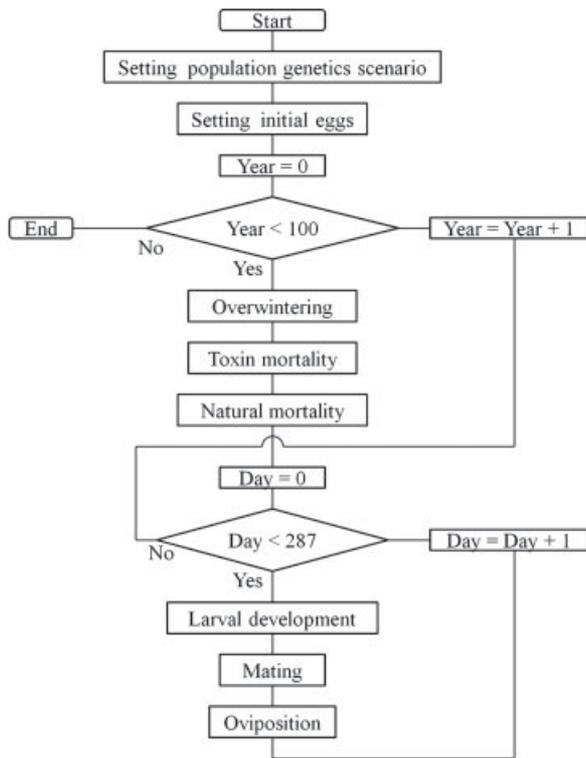
Figure 1. Processes in the *D. v. virgifera* model.

more than the other sex, the sex ratios of beetles emerging in *Bt* and non-*Bt* corn plots will differ. The authors used a simulation model to study the effects of (1) temporal separation between male and female beetle emergence from *Bt* and non-*Bt* corn and (2) the skewed sex ratio of beetles emerging from *Bt* corn on evolution of *Bt* resistance in *D. v. virgifera*.

2 EXPERIMENTAL METHODS

2.1 Simulation model

The overall algorithm of this model is shown in Fig. 1. The model is programmed in Microsoft Visual C++. The model's spatial design, population genetics scenarios, toxin mortality and the parameters

of *D. v. virgifera* biology are described in the subsections that follow. Parameter sensitivity analysis was used to study the effect of (1) the proportion of non-transgenic corn in the seed blend ($propPlt_{non-Bt}$), (2) toxin mortality ($maxStox$, $minStox$, h), (3) a skew in toxin mortality towards one sex (z) and (4) maturation delay for individuals emerging from *Bt* corn ($maxDel_{male}$, $minDel_{male}$, $hDel_{male}$, $maxDel_{female}$, $minDel_{female}$, $hDel_{female}$) on evolution of resistance by *D. v. virgifera* to *Bt* corn. The ranges of values for parameters for sensitivity analyses are shown in Table 1.

2.2 Spatial design

In the model, *Bt* and non-*Bt* corn seeds were planted annually as a seed blend in a 100 ha field: 79 000 seeds were planted per hectare. The proportions of *Bt* and non-*Bt* corn were $propPlt_{Bt}$ and $propPlt_{non-Bt}$ respectively. The standard value for the proportion of the refuge (i.e. non-*Bt* corn) ($propPlt_{non-Bt}$) was 0.2 with single-trait *Bt* corn and 0.05 with pyramided *Bt* corn. Seed blends generally permit better mixing of adult *D. v. virgifera* genotypes than block refuges.[6]

Modeling results obtained by Pan *et al.*[18] and data presented by Binning *et al.*[19] indicate that modeling a seed blend as a randomly distributed refuge resulting in random mating may be a reasonable simplification when studying resistance evolution by *D. v. virgifera*. Thus, the authors did not explicitly model larval movement in the present model so that they could focus on parameters pertaining to adult beetles. It is difficult to predict how larval behavior and survival influence insect resistance management owing to interactions between larval behavior and survival.[20] Note that larval movement hastens resistance evolution if the heterozygotes have a selective advantage over the homozygous susceptible in larval movement, but this is not always the case.[6,18−21]

2.3 Population genetics and toxin mortality

For simplicity, two unlinked, diallelic autosomal loci in *D. v. virgifera* were assumed to determine resistance to transgenic corn expressing *Bt* toxins. The first and the second loci were assumed to provide resistance to the first and the second *Bt* toxin respectively. Therefore, these loci determined the first ($stox1_{p,g}$) and the second toxin survival rate ($stox2_{p,g}$) respectively, where plant type and genotype of insect are denoted by p and g respectively.

D. v. virgifera is not very sensitive to Cry3Bb1, Cry34Ab1/Cry35Ab1 and mCry3A proteins, and older larvae

		Range for sensitivity	
Parameter	Standard value	analysis	Description
$propPlt_{non-Bt}$	0.2 (single-toxin *Bt* corn), 0.05 (pyramided *Bt* corn)	0.2−0.5 (single-toxin *Bt* corn), 0.05−0.2 (pyramided *Bt* corn)	The proportion of refuge of non-*Bt* corn
$minStox$	0.01	0.001−0.01	The minimum toxin survival rate
h	0.5	0.01−1	The dominance of a resistance allele
$maxStox$	1	0.25−1	The maximum toxin survival rate
z	−0.5	(−0.9)−0.9	The parameter determines the distribution of toxin survival between the two sexes
$maxDel_{male}$	3 days	0−28	The greatest degree of delay for adult emergence of males in *Bt* corn
$hDel_{male}$	0.5	0−1	The dominance of the resistance allele for delayed adult emergence of males in *Bt* corn
$minDel_{male}$	0 days	0−28	The smallest degree of delay for adult emergence of males in *Bt* corn
$maxDel_{female}$	2 days	0−28	The greatest degree of delay for adult emergence of females in *Bt* corn
$hDel_{female}$	0.5	0−1	The dominance of the resistance allele for delayed adult emergence of females in *Bt* corn
$minDel_{female}$	0 days	0−28	The smallest degree of delay for adult emergence of females in *Bt* corn

Table 1. List of parameters for sensitivity analyses

can survive the exposure to *Diabrotica*-active *Bt* corn.[22] Transgenic corn expressing Cry3Bb1 does not effectively control larvae older than the first instar.[22] For this reason, toxin mortality was applied before density-dependent mortality occurred. The toxin survival rate for homozygous resistant larvae (R1R1 for the single-toxin case and R1R1—R2R2 for the pyramided-toxin case) has not been evaluated. It was assumed that homozygous resistant larvae had a toxin survival rate, *maxStox*, of 1 for each insecticidal trait, which means that no mortality occurred among homozygous resistant larvae. The effect of the maximum toxin survival rate on resistance evolution was studied with a range of *maxStox* from 0.25 to 1. The survival rate for homozygous susceptible larvae was *minStox*. Traits eCry3.1Ab, Cry34Ab1/Cry35Ab1, Cry3Bb1 and mCry3A caused 99.97, 96.48, 98.49 and 97.83% reductions in beetle emergence relative to that from isoline corn respectively.[11,12,23] The standard value for *minStox* was set at 0.01 to reflect these data. If larvae were heterozygous at the first locus (R1S1) or at the second locus (R2S2), *stox1* and *stox2* were calculated by equations 1 and 2 respectively:

$$stox1_{p,R1S1} = (maxStox \times h) + [minStox \times (1 - h)] \quad (1)$$

$$stox2_{p,R2S2} = (maxStox \times h) + [minStox \times (1 - h)] \quad (2)$$

where *h* is the dominance of a resistance allele (the standard value for *h* was 0.5), and *stox1* is the toxin survival rate in the single-toxin case.

Pyramided *Bt* corn expressing mCry3A and eCry3.1Ab caused a 99.91% reduction in beetle emergence relative to emergence from isoline corn.[11] This emergence reduction was greater than the multiplication of the two toxin mortalities caused solely by eCry3.1Ab or mCry3A. The hybrid of DAS59122-7 (Cry34Ab1/Cry35Ab1) and MON88017 (Cry3Bb1) resulted in a 1.8% toxin survival rate, which was greater than the product of the two toxin survival rates attributable solely to each parental event (i.e. MON88017 and DAS59122-7).[11] For this study, the toxin survival rate for larvae in pyramided *Bt* corn was the minimum of the two toxin survival rates (i.e. *stox1* and *stox2*) determined by the first and the second locus.[24]

2.4 Sex-specific toxin mortality

The sex ratio of *D. v. virgifera* is variable from year to year and is not significantly affected by egg density; the effect of insecticides on the sex ratio has been inconsistent.[25] Field and greenhouse studies showed that the proportion of female beetles emerging from *Bt* corn was greater than that of female beetles emerging from non-*Bt* corn.[15,16] Oyediran *et al.*[16] reported that the proportion of female beetles emerging from *Bt* corn expressing Cry3Bb1 (91.7%) was greater than that of female beetles emerging from isoline corn (44.7%) at a Missouri field site in 2003. However, the difference between the proportion of female beetles emerging in *Bt* corn (83.78%) and that in isoline corn (70.62%) was not significant at the same site in 2004. Equations 3, 4 and 5 were used to determine sex-specific toxin survival rates, *sstox*. For gender *x*, plant type *p* and genotype *g*, the authors accounted for potential differences in mortality that produce a variety of sex ratios in adults, using the following functions:

$$sstox = stox_{p,g,x} \quad \text{if } stox_{p,g} > 0.5 \quad (3)$$

$$sstox_{p,g,male} = stox_{p,g} + (z_{p,g} \times stox_{p,g}) \quad \text{if } 0 \leq stox_{p,g} \leq 0.5 \quad (4)$$

$$sstox_{p,g,female} = stox_{p,g} - (z_{p,g} \times stox_{p,g}) \quad \text{if } 0 \leq stox_{p,g} \leq 0.5 \quad (5)$$

where *sstox* is less than or equal to 1:

$$-1 < z_{p,g} \leq 1 \quad (6)$$

It was assumed that the proportion of females at the embryonic stage was 0.5. The sex ratio of beetles emerging from *Bt* corn was different from that of beetles from non-*Bt* corn if *Bt* toxins killed one sex more than the other sex. The parameter $z_{p,g}$ determined the distribution of toxin survival between the two sexes. Equation 6 controlled a range of $z_{p,g}$. The total number of insects controlled by toxins was not affected by $z_{p,g}$. When $z_{p,g}$ was greater than 0, the toxin survival rate for males was greater than that for females, which meant that the toxin killed more females than males. The toxin survival rate for females was greater than that for males if $z_{p,g}$ was less than 0. If $z_{p,g}$ was −1 or 1, only larvae of one sex survived. The standard value for *z* was chosen to be −0.5, which was based on data in Oyediran *et al.*[16] The number of young larvae was calculated by the following equations:

$$lar_{p,g,male} = egg_{p,g} \times sstox_{p,g,male} \times (1 - pf) \quad (7)$$

$$lar_{p,g,female} = egg_{p,g} \times sstox_{p,g,female} \times pf \quad (8)$$

2.5 Natural survival rate

Density-dependent mortality was assumed to occur after toxin mortality:

$$oLar_{p,g,x} = lar_{p,g,x} \times SurRat_p \quad (9)$$

where $oLar_{p,g,x}$ is the number of larvae after density-dependent mortality, and $SurRat_p$ is the natural survival rate.

Hibbard *et al.*[23] showed that the density-dependent effect on egg-to-adult survival rate was significant if the number of eggs per 30.5 cm of row was greater than 800. The unit of egg density was converted by using the description of an experiment in Hibbard *et al.*[23] to calculate a density-independent survival rate by using equations 10 and 11. The unit of egg density was converted from the number of eggs per 30.5 cm of row to the number of eggs per plant by multiplying 0.625 {= [(3.05 m/0.305 m) × 4 rows]/64 plants} by the number of eggs per 30.5 cm of row. If the number of eggs per corn plant was equal to or greater than 500 (= 800 × 0.625), survival rate was calculated by equation 11. Equation 11 was derived by applying a negative exponential model (model: $y = a \times e^{bx}$, $P = 0.01$, $R^2 = 0.39$; *a*: $t = 7.04$, $P < 0.05$; *b*: $t = -2.52$, $P < 0.05$) to data in Fig. 4 from Hibbard *et al.*:[23]

$$SurRat_p = 0.0519 \quad \text{if } \frac{\Sigma_g \Sigma_x lar_{p,g,x}}{7\,900\,000 \times propPlt_p} < 500 \quad (10)$$

$$SurRat_p = 0.0513 \times e^{-0.0006 \times \frac{\Sigma_g \Sigma_x lar_{p,g,x}}{7\,900\,000 \times propPlt_p}}$$

$$\text{if } \frac{\Sigma_g \Sigma_x lar_{p,g,x}}{7\,900\,000 \times propPlt_p} \geq 500 \quad (11)$$

where $\frac{\Sigma_g \Sigma_x lar_{p,g,x}}{7\,900\,000 \times propPlt_p}$ is the total number of larvae per plant after *Bt* toxin mortality, and 7 900 000 is the number of corn plants per 100 ha.

2.6 Maturation delay

The authors used data from Hibbard *et al.*[11] and Murphy *et al.*[13] to analyze the temporal pattern of beetle emergence. In this model, parameters regarding maturation delay were based on the emergence of beetles from *Bt* corn expressing Cry3Bb1. Pan

et al.[18] modeled maturation delay in *D. v. virgifera* emerging from *Bt* corn expressing Cry34Ab1/Cry35Ab1. The cumulative distribution function (CDF) of the normal distribution, $F(t; \mu, \sigma^2)$, was used to depict the temporal pattern of emergence of male or female beetles from *Bt* and non-*Bt* corn, where t, μ and σ^2 are the time, the center of temporal distribution of beetle emergence and the variance of temporal distribution of beetle emergence respectively. The difference in parameter estimates, which were μ and σ^2, was tested by using dummy variables. Function nlinfit in Matlab® was used for the nonlinear regression analyses. The analyses regarding beetle emergence are shown in the supporting information.

Based on the results from the analyses, *Bt* toxins were assumed to affect the mean emergence time, but the standard deviation of emergence time was assumed not to be influenced by *Bt* toxins. For this model, the mean emergence times for male and female beetles from non-*Bt* corn were the averages of the mean emergence times for male and female beetles from transgenic corn not expressing Cry3Bb1 in 2007 and 2008 (male beetle: Julian day 201.91; female beetle: Julian day 209.83). The proportion of male and female beetles emerging per day was calculated once per day by the following equations:

$$propA_{p,g,male}(t) = F[t, (201.91 + Del_{p,g,male}), 8.33^2]$$
$$- F[(t-1), (201.91 + Del_{p,g,male}), 8.33^2] \quad (12)$$

$$propA_{p,g,female}(t) = F[t, (209.83 + Del_{p,g,female}), 11.11^2]$$
$$- F[(t-1), (209.83 + Del_{p,g,female}), 11.11^2] \quad (13)$$

where $propA_{p,g,x}(t)$ is the proportion of a sex x of a genotype g of beetles emerging in a type of plant p at time t, and $Del_{p,g,x}$ is the delay in adult emergence of a sex x of a genotype of larvae g in a type of plant p.

The standard deviations of emergence time for male and female beetles were the averages of the standard deviations of emergence time for male and female beetles from transgenic corn expressing Cry3Bb1 and transgenic corn not expressing Cry3Bb1 in 2007 and 2008 (male beetle: 8.33 days; female beetle: 11.11 days). Figure 2 shows the time shift of beetle emergence in this model.

2.7 Population genetics scenarios for maturation delay

The standard values for parameters regarding the maturation delay were based on Murphy *et al.*[13] The most susceptible genotypes to *Bt* toxins (i.e. S1S1 in the one-toxin case and S1S1|S2S2 in the two-toxin case) were assumed to show the greatest degree of delay for adult emergence (one-toxin case: $Del_{Bt1,S1S1,male} = maxDel_{male}$, $Del_{Bt1,S1S1,female} = maxDel_{female}$; two-toxin case: $Del_{Bt2,S1S1|S2S2,male} = maxDel_{male}$, $Del_{Bt2,S1S1|S2S2,female} = maxDel_{female}$). The standard value of $maxDel_{male}$ was 3, which is the average of the differences between the mean emergence time for male beetles from transgenic corn expressing Cry3Bb1 and the one not expressing Cry3Bb1 in 2007 and 2008. The standard value of $maxDel_{female}$ was 2, which is the average of the differences between the mean emergence time for female beetles from transgenic corn expressing Cry3Bb1 and the one not expressing Cry3Bb1 in 2007 and 2008. The most resistant genotypes to *Bt* toxins, which are R1R1 in the single-toxin case and R1R1|R2R2 in the pyramided-toxin case, were assumed to show the smallest degree of delay for adult emergence (single-toxin case: $Del_{Bt1,R1R1,male} = minDel_{male}$, $Del_{Bt1,R1R1,female} = minDel_{female}$;
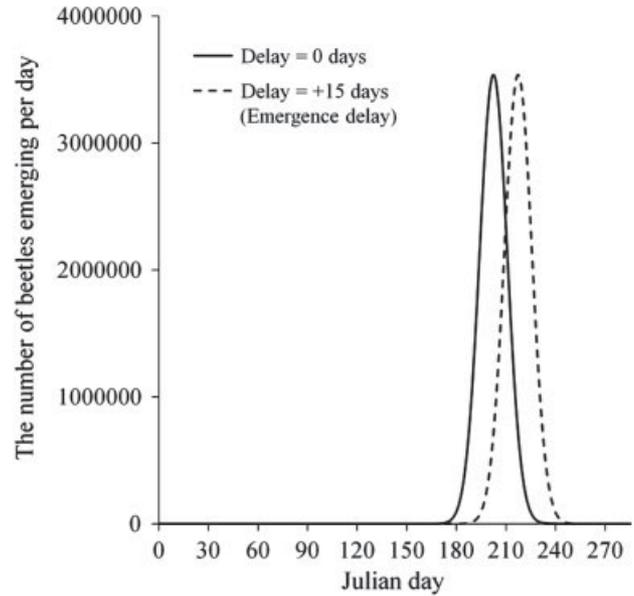

Figure 2. Time shift in beetle emergence from *Bt* corn in the *D. v. virgifera* model.

pyramided-toxin case: $Del_{Bt2,R1R1|R2R2,male} = minDel_{male}$, $Del_{Bt2,R1R1|R2R2,female} = minDel_{female}$). The standard values of $minDel_{male}$ and $minDel_{female}$ were 0. An intermediate degree of delay for adult emergence was assumed to occur for genotypes that were moderately resistant to *Bt* toxins (single-toxin case: $Del_{Bt1,R1S1,male} = midDel_{male}$, $Del_{Bt1,R1S1,female} = midDel_{female}$; pyramided-toxin case: $Del_{Bt2,R1S1|R2S2,male} = Del_{Bt2,R1R1|R2S2,male} = Del_{Bt2,R1S1|R2R2,male} = Del_{Bt2,R1S1|S2S2,male} = Del_{Bt2,S1S1|R2S2,male} = midDel_{male}$, $Del_{Bt2,R1S1|R2S2,female} = Del_{Bt2,R1R1|R2S2,female} = Del_{Bt2,R1S1|R2R2,female} = Del_{Bt2,R1S1|S2S2,female} = Del_{Bt2,S1S1|R2S2,female} = midDel_{female}$). $midDel_{male}$ and $midDel_{female}$ were calculated by the following equations:

$$midDel_{male} = (minDel_{male} \times hDel_{male})$$
$$+ [maxDel_{male} \times (1 - hDel_{male})] \quad (14)$$

$$midDel_{female} = (minDel_{female} \times hDel_{female})$$
$$+ [maxDel_{female} \times (1 - hDel_{female})] \quad (15)$$

where $hDel_{male}$ and $hDel_{female}$ are the dominance of the resistance allele for delayed adult emergence of males and females in *Bt* corn respectively (the standard values for $hDel_{male}$ and $hDel_{female}$ were 0.5).

Several scenarios of time shift for adult emergence from *Bt* corn were studied by changing the values for $maxDel_{male}$, $maxDel_{female}$, $minDel_{male}$, $minDel_{female}$, $hDel_{male}$ and $hDel_{female}$. The range of $maxDel_{male}$, $maxDel_{female}$, $minDel_{male}$ and $minDel_{female}$ for sensitivity analyses ranged from 0 to 28 days. The effect of great emergence delays (>14 days) on resistance evolution was studied for theoretical purposes. The time shift for beetle emergence was assumed not to occur for all genotypes emerging from non-*Bt* plants ($Del_{non-Bt,g,x} = 0$). The number of newly emerging beetles was calculated by the following equation:

$$adt_{p,g,x,0} = propA_{p,g,x}(t) \times oLar_{p,g,x} \times SurRat_p \quad (16)$$

where $SurRate_p$ is the natural survival rate of larvae on a type of plant p, $adt_{p,g,x,a}$ is the number of beetles of a sex x of a genotype

g at age a emerging from a type of plant p, $propA_{p,g,x}(t)$ is the proportion of a sex x of a genotype g of beetles emerging in a type of plant p at time t, and $oLar_{p,g,x}$ is the number of larvae after density-dependent mortality.

2.8 Female beetles

Female beetles typically mate only once, frequently within 24 h of emergence while still light-colored teneral adults.[26,27] Quiring and Timmins[28] studied the proportion of mated teneral female beetles by counting the number of mating pairs in field cages at two fields in Harrow and Elora, Ontario, Canada. Spermatophores in the bursa copulatrix of female beetles were checked by dissection to confirm that mating had occurred because spermatophores are distinguishable in the bursa copulatrix of female beetles for approximately 5–7 days after mating.[29] The data from Quiring and Timmins[28] were pooled across fields to calculate the proportion of mating teneral female beetles. The proportions of teneral and non-teneral female beetles with spermatophores were 46.58 and 83.23% respectively. Female beetles remain teneral for 24 h after emergence.[26] For this reason, teneral female and non-teneral female beetles were considered to be 0–1 days old and greater than 1 day old respectively. The proportions of 1-day-old and 2-day-old virgin female beetles mating [$propM(a)$] were chosen to be 0.4658 and 0.5342 respectively. Accordingly, 100% of virgin female beetles were assumed to have mated by 2 days after emergence. Values of $propM(a)$ were calculated daily. If a was greater than 2, then $propM(a)$ was 0.

2.9 Male beetles

Decline in the mating ability of male beetles owing to age can affect resistance evolution by *D. v. virgifera* if beetle emergence from *Bt* and non-*Bt* corn is not synchronous.[30] The authors used data from Quiring and Timmins[28] to build a model for calculating the probability of mating for male beetles at a particular age. In the Quiring and Timmins study,[28] one male and two young, virgin female beetles were placed in a screen-covered cage. The two female beetles were replaced with two new, young, virgin female beetles daily. Young corn leaves, ears and tassels were provided in cages for the first 6 days, and thereafter solely young leaves were provided for food. These data were chosen for two reasons. Firstly, the sex ratio of males and virgin females in their field cages was female skewed. Secondly, the diet provided to males was similar to the diet that male beetles utilize in the field. Corn ears and tassel material are available to male beetles in the field for a limited period. Data points shown in Fig. 8a from Quiring and Timmins were acquired by using Engauge Digitalizer 4.1, and were analyzed using linear regression (model: $F = 26.36$, $P < 0.01$, $R^2 = 0.39$; slope: $t = -5.13$, $P < 0.01$; intercept: $t = 8.46$, $P < 0.01$) (Fig. 3).[28] Equation (17) was used to calculate the probability of a mating male:

$$maleP(a) = -0.0123 \times a + 0.5122 \qquad (17)$$

where $maleP(a)$ is the probability of mating male beetles at age a. If $maleP(a)$ calculated by equation (17) was less than 0, then $maleP(a)$ was 0.

Equation (18) was used daily to calculate the proportion of a genotype g of mating male beetles, $propP_g$:

$$propP_g = \frac{\Sigma_p \Sigma_{a=0}^{32} maleP(a) \times adt_{p,g,male,a}}{\Sigma_p \Sigma_g \Sigma_{a=0}^{32} maleP(a) \times adt_{p,g,male,a}} \qquad (18)$$
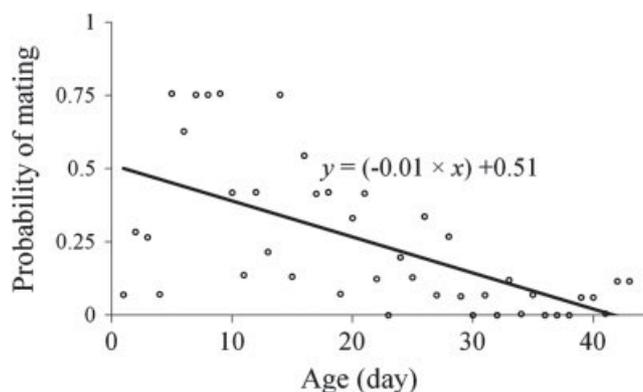

Figure 3. The probability of *D. v. virgifera* males mating at different ages.[28]

where $adt_{p,g,x,a}$ is the number of beetles of a sex x of a genotype g of beetles at age a emerging from a type of plant p.

The maximum age for mating for male beetles was 42 days, which was estimated by using equation (17) with the estimated parameters. The indices pg and p denote a paternal genotype and a plant type respectively. The age of adults, which is a in $adt_{p,g,x,a}$, increased daily by 1.

2.10 Mating

A male beetle emerging from either a *Bt* or a non-*Bt* plant in a seed blend was assumed to have an equal chance of mating with a female beetle emerging from a non-*Bt* or a *Bt* plant. Equation (19) was used daily to calculate a genotype mg of female beetles mating with a genotype pg of male beetles and laying a genotype g of eggs:

$$mtFem_{g,a} = \sum_p \sum_{pg} \sum_{mg} probOff_{g,pg,mg} \times propP_{pg} \times propM(a)$$
$$\times adt_{p,mg,female,a} \qquad (19)$$

where $mtFem_{g,a}$ is the number of mated female beetles of a genotype g at age a (the age of mated female beetles increased daily by 1), $probOff_{g,pg,mg}$ is the probability of offspring of a genotype g derived from female beetles of a genotype mg mating with male beetles of a genotype pg (a Mendelian function was used to calculate $probOff_{g,pg,mg}$) $propp_{pg}$ is the proportion of a genotype pg of mating male beetles, and $propM(a)$ is the proportion of mating female beetles at age a.

2.11 Oviposition

The mean duration of the pre-oviposition period estimated by Branson and Johnson[31] is 14.3 days. The time step for oviposition was 1 day for this model; the pre-oviposition period for this model was chosen to be 14 days:

$$propOV(a) = 0 \text{ if } a < 15 \qquad (20)$$

where $propOV(a)$ is the proportion of oviposition by female beetles at age a.

Branson and Johnson studied oviposition of field-collected *D. virgifera* in the laboratory.[31] The relationship between the proportion of eggs laid per female every 10 days and the age of a female beetle at the midpoint of this period was described by a linear model shown in equation (21) (model: $F = 55.56$,
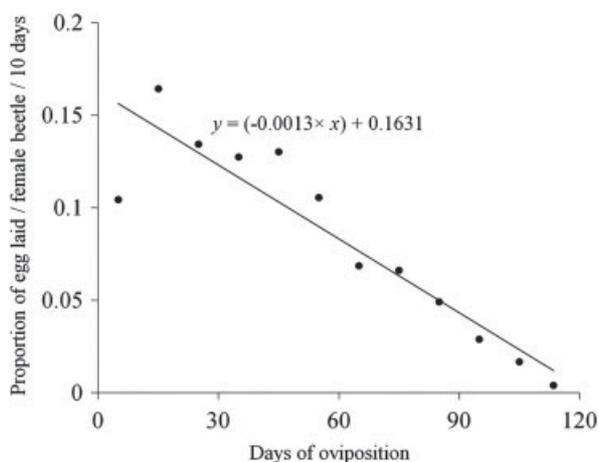
Figure 4. The proportion of eggs laid by a female *D. v. virgifera*.[31]

$P < 0.01$; slope: $t = -7.45$, $P < 0.01$; intercept: $t = 13.22$, $P < 0.01$) (Fig. 4):

$$propOV\,(a) = [-0.0013 \times (a - 15) + 0.1631]$$
$$\times\, 0.1 \times 0.9935 \;\; \text{if } a \geq 15 \qquad (21)$$

where 0.9935 is a scaling factor to make the total proportion of eggs laid by a female beetle.

If $propOV(a)$ was less than 0, $propOV(a)$ was set to 0. Because the number of eggs laid per female beetle every 10 days was divided by the total number of eggs laid, 0.1 was multiplied with the linear model to derive the daily proportion. Equation (21) attributes a greater proportion of oviposition to old female beetles than does the daily oviposition function [$6 \times (t - 13) \times e^{-0.115 \times (t - 13)}$] used in Onstad *et al.*[32] The fecundity for this study and Onstad *et al.* was 440, which was the average number of viable eggs laid by a female beetle throughout its lifetime under realistic nutritional conditions.[32,33] The number of eggs laid by female beetles was calculated by the following equation:

$$egg_{p,g} = (propPlt_p \times \Sigma_{a=1}^{122}\,propOV\,(a) \times 440 \times mtFem_{g,a} \quad (22)$$

where $egg_{p,g}$ is the genotype g of eggs on a type of plant p. According to equation (21), the maximum age of female beetles ovipositing was 122 days.

Table 2. Durability of *Bt* corn (in years) as influenced by the initial allele frequencies and the proportion of non-*Bt* corn in the seed blend ($propPlt_{non-Bt}$). The maximum toxin survival rate ($maxStox$) was 1. The minimum toxin survival rate ($minStox$) was 0.01. The dominance of a resistance allele (h) was 0.5

Bt corn	$propPlt_{non-Bt}$	10^{-5}	$10^{-4\,a}$	10^{-3}	10^{-2}
One toxin	0.2[a]	14	11	9	6
	0.3	20	16	12	8
	0.4	31	25	19	12
	0.5	47	38	28	19
Two toxins	0.05[a]	28	22	16	10
	0.1	36	28	20	13
	0.2	60	46	32	18

[a] Standard values.

Table 3. Durability of single-toxin *Bt* corn (in years) as influenced by the maximum toxin survival rate ($maxStox$), the dominance of a resistance allele (h) and the proportion of non-*Bt* corn in the seed blend ($propPlt_{non-Bt}$). The minimum toxin survival rate ($minStox$) was 0.01

			$maxStox$		
$propPlt_{non-Bt}$	h	0.25	0.5	0.75	1^{a}
0.2[a]	0.1	100	51	36	28
	0.25	51	27	20	16
	0.5[a]	31	18	13	11
	0.75	24	14	11	10
	1.0	20	12	10	8
0.3	0.1	>100	93	61	46
	0.25	96	47	32	24
	0.5	59	29	20	16
	0.75	45	23	16	12
	1.0	37	19	13	11
0.5	0.1	>100	>100	>100	>100
	0.25	>100	>100	80	60
	0.5	>100	75	49	38
	0.75	>100	58	39	30
	1.0	99	50	33	26

[a] Standard values.

Oviposition continues until the time of frost.[34] First fall frost dates at Springfield, Illinois, South Bend, Indiana, and Cedar Rapids, Iowa, in 2011 were 13 October, 19 October and 6 October respectively (National Climatic Data Center, http://lwf.ncdc.noaa.gov/oa/ncdc.html). The average of these three dates is Julian day 287, which was the date on which oviposition stopped for this model. Eggs laid in the late summer and fall do not hatch until the following spring.[34] Eggs overwinter in the soil, and they hatch through late May and early June.[1] Onstad *et al.*[32] analyzed overwintering rates reported in Godfrey *et al.*,[35] and concluded that the overwintering egg survival rate was 50%:

$$egg_{p,g} = egg_{p,g} \times 0.5 \qquad (23)$$

2.12 Initial population

The standard values for the initial frequencies of the resistance alleles were 10^{-4}. The initial egg genotypic frequencies followed a Hardy–Weinberg distribution. The average number of beetles per corn plant in northern and central Illinois, which were at risk of potential *D. v. virgifera* injury in first-year corn, was 0.17 in 2011.[36] Thus, the initial density of eggs per corn plant in standard simulations was 37.4 [= 0.17 the number of adults per corn plant $\times$ 0.5 (the proportion of female beetles) $\times$ 440 (the number of eggs laid by a female beetle)]. The average numbers of beetles per corn plant in Lee and McLean counties were 0.64 and 0.81 in 2012.[37] The economic threshold (beetles per plant) for first-year cornfields is 0.5, and the economic threshold for continuous corn production systems is 0.75–1.0 beetles per plant.[38] The initial number of eggs per corn plant ranged from 20 to 320 in a sensitivity analysis. Each year lasted 287 days from 1 January to 13 October. Simulations lasted 100 years.

3 RESULTS

Resistance was considered to evolve if the frequency of a resistance allele exceeded 0.5. Durability in this study was defined as the

Table 4. Durability of pyramided *Bt* corn (in years) as influenced by the minimum toxin survival rate (*minStox*), the dominance of a resistance allele (*h*) and the proportion of non-*Bt* corn in the seed blend (*propPlt$_{non-Bt}$*). The maximum toxin survival rate (*maxStox*) was 1

propPlt$_{non-Bt}$	h	minStox	
		0.001	0.01[a]
0.05[a]	0.01	>100	>100
	0.1	82	>100
	0.25	33	35
	0.5[a]	22	22
	0.75	18	18
	1.0	15	15
0.1	0.01	>100	>100
	0.1	>100	>100
	0.25	51	56
	0.5	28	28
	0.75	21	21
	1.0	17	18
0.2	0.01	>100	>100
	0.1	>100	>100
	0.25	>100	>100
	0.5	45	46
	0.75	28	29
	1.0	22	23

[a] Standard values.

Table 5. Durability of pyramided *Bt* corn (in years) as influenced by the maximum toxin survival rate (*maxStox*), the dominance of resistance alleles (*h*) and the proportion of non-*Bt* corn in the seed blend (*propPlt$_{non-Bt}$*). The minimum toxin survival rate (*minStox*) was 0.01

propPlt$_{non-Bt}$	h	maxStox			
		0.25	0.5	0.75	1[a]
0.05[a]	0.1	>100	>100	>100	>100
	0.25	>100	84	51	35
	0.5[a]	>100	41	29	22
	0.75	85	30	23	18
	1.0	63	25	19	15
0.1	0.1	>100	>100	>100	>100
	0.25	>100	>100	97	56
	0.5	>100	62	38	28
	0.75	>100	39	27	21
	1.0	85	30	22	18
0.2	0.1	>100	>100	>100	>100
	0.25	>100	>100	>100	>100
	0.5	>100	>100	78	46
	0.75	>100	80	40	29
	1.0	>100	47	29	23

[a] Standard values.

number of years required for the frequency of a resistance allele to increase from the initial allele frequency to 0.5.

3.1 Single-toxin *Bt* corn

The effect of toxin survival rates and refuge on resistance evolution was studied with maturation parameters at their standard settings as described in Table 1. In a range of initial resistance allele frequency from 10^{-5} to 10^{-3}, resistance to single-toxin *Bt* corn evolved within 20 years when the proportion of non-*Bt* corn in the seed blend was less than or equal to 0.3 and the rest of the parameters were at their standard setting (Table 2). Resistance to single-toxin *Bt* corn evolved within 13 years when the dominance of the resistance allele (*h*) was greater than or equal to 0.5, the maximum toxin survival rate (*maxStox*) was greater than or equal to 0.75 and the proportion of refuge in the seed blend was 20% (Table 3). If the maximum toxin survival rate was 1, the durability of single-toxin *Bt* corn was 0–2 years greater when the minimum toxin survival rate (*minStox*) was 0.01 than when the minimum toxin survival rate was 0.001. If the dominance of the resistance allele was greater than or equal to 0.5 and the proportion of refuge in the seed blend was 20%, resistance of *D. v. virgifera* to *Bt* corn
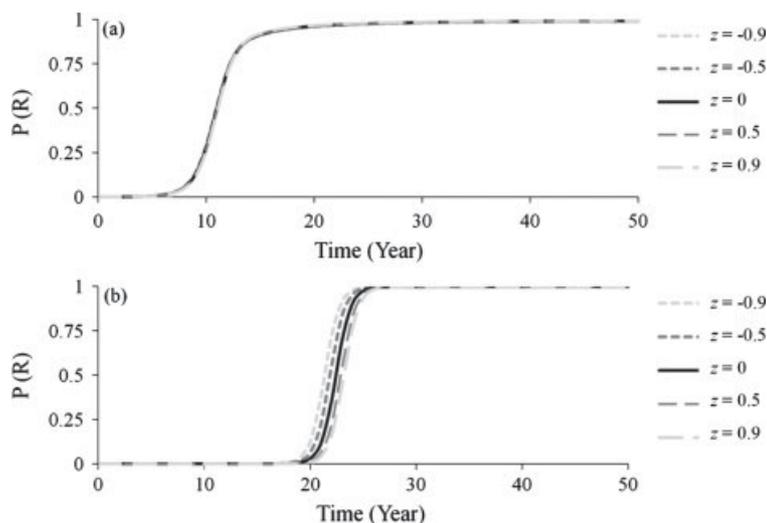

Figure 5. The effect of sex-specific toxin mortality on evolution of resistance by *D. v. virgifera* to transgenic corn expressing (a) a single *Bt* toxin and (b) two *Bt* toxins. The parameter *z* determines the distribution of toxin mortality between the two sexes, where a value of −1 means only females survive and a value of 1 means only males survive.
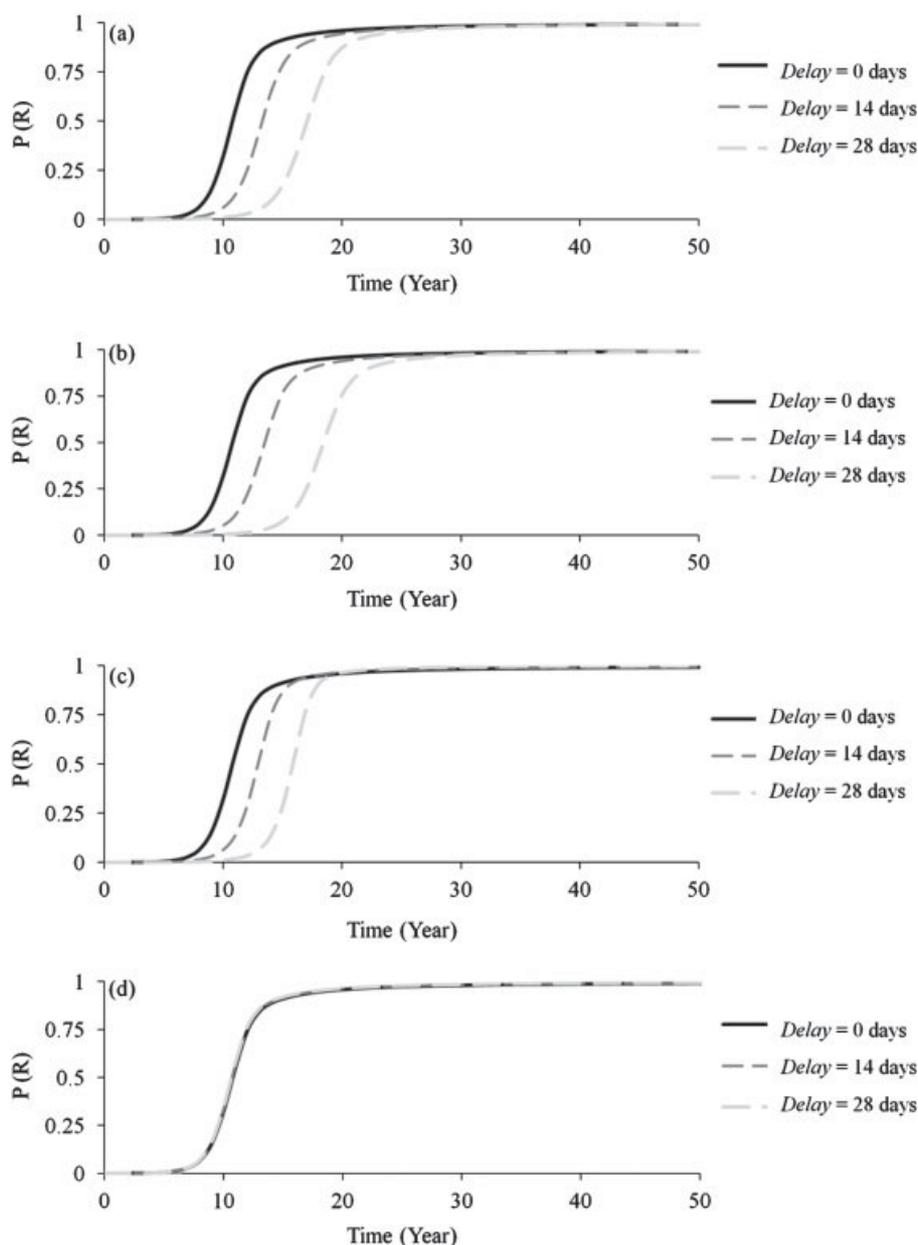
Figure 6. The effect of a time shift in the emergence of beetles from transgenic corn expressing a single *Bt* toxin with 20% refuge-in-a-bag on resistance allele frequency in the population [P(R)]. (a) The effect of a time shift in the emergence of beetles of all genotypes from single-toxin *Bt* corn. (b) The effect of a time shift in the emergence of homozygous resistant and heterozygous beetles from single-toxin *Bt* corn. (c) The effect of a time shift in the emergence of heterozygous and homozygous susceptible beetles from single-toxin *Bt* corn. (d) The effect of a time shift in the emergence of homozygous susceptible beetles from single-toxin *Bt* corn. If *Delay* is greater than 0, there is an emergence delay. There is early emergence if *Delay* is less than 0.

expressing one toxin evolved within 11 years. In a 50% seed blend, it took 26 years for resistance to one *Bt* toxin to evolve when the minimum toxin survival rate was 0.001 and the dominance of the resistance allele was 1. The duration for the resistance allele frequency to increase from 3 to 50% was studied to estimate the effect of the proportion of blended refuge on the time lapse between the time the resistance is first detected and the time the resistance allele frequency exceeds 0.5. In a single-toxin case, depending on the minimum toxin survival rate and the dominance of the resistance allele, it took approximately 4 years, 5 to 10 years and 15 to >24 years for the resistance allele frequency to increase from 3 to 50% when the proportion of blended refuge was 20, 30 and 50% respectively.

The evolution of resistance to single-toxin *Bt* corn was not sensitive to the density of the initial population. When all parameters were at their standard setting, resistance to single-toxin *Bt* corn evolved 1 year earlier when the initial number of eggs per corn plant was 20 than when the initial number of eggs per corn plant was 320.

3.2 Pyramided *Bt* corn
Resistance to pyramided *Bt* corn did not evolve within 20 years in a 5% seed blend when the initial resistance allele frequencies were less than or equal to 1.0×10^{-4} and the remaining parameters were held at their standard setting. Resistance to pyramided *Bt* corn evolved within 22 years if the dominance
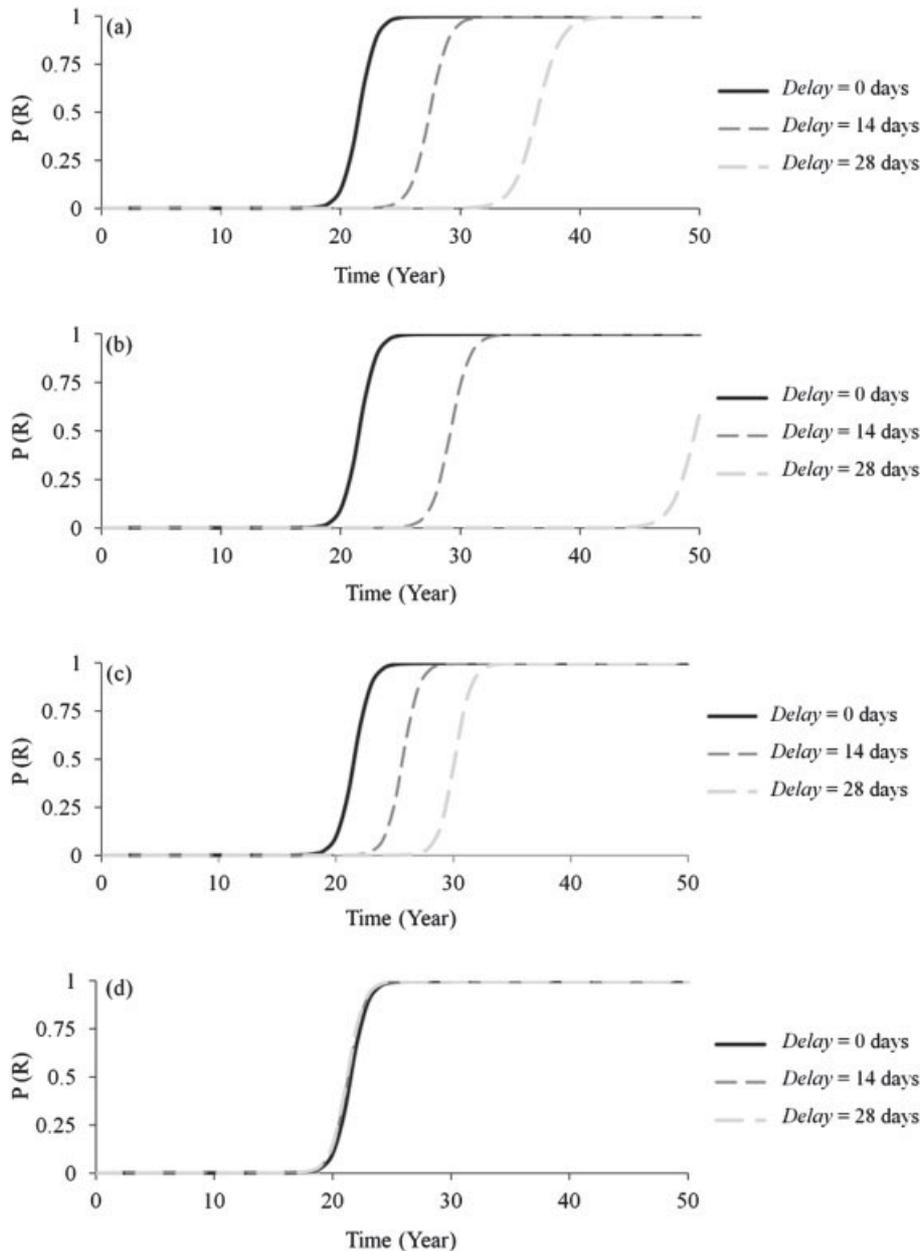
Figure 7. The effect of a time shift in the emergence of beetles from transgenic corn expressing two *Bt* toxins with 5% refuge-in-a-bag on resistance allele frequency in the population [P(R)]. (a) The effect of a time shift in the emergence of beetles of all genotypes from pyramided *Bt* corn. (b) The effect of a time shift in the emergence of homozygous resistant and heterozygous beetles from pyramided *Bt* corn. (c) The effect of a time shift in the emergence of heterozygous and homozygous susceptible beetles from pyramided *Bt* corn. (d) The effect of a time shift in the emergence of homozygous susceptible beetles from pyramided *Bt* corn. If *Delay* is greater than 0, there is an emergence delay. There is early emergence if *Delay* is less than 0.

of a resistance allele (*h*) was greater than or equal to 0.5 and the proportion of non-*Bt* corn in the seed blend was 5% (Tables 4 and 5). It took approximately 3 years for the resistance allele frequencies for two *Bt* toxins to increase from 0.03 to 0.5 when the proportion of non-*Bt* corn in the seed blend was 5%, while it took 5 years when the proportion of non-*Bt* corn in the seed blend was 20%.

The evolution of resistance to pyramided *Bt* corn was not sensitive to the density of the initial population. When all parameters were at their standard setting, resistance to pyramided *Bt* corn evolved in 22 years in a range of initial number of eggs per corn plant of 20–320.

3.3 Sex-specific toxin survival
Resistance evolution was not significantly influenced by the susceptibility of the sexes to *Bt* toxins (Fig. 5). When *z*, which determined the distribution of toxin survival between the two sexes, was greater than 0, the toxin survival rate for males was greater than that for females, which means that the toxin killed more females than males. Resistance evolved more rapidly when males were more susceptible to *Bt* toxin than females ($z < 0$). The difference between the durability of *Bt* toxin with $z = -0.9$ and that with $z = 0$ was 0 years in the single-toxin case and 1 year in the pyramided-toxin case. Resistance evolved more slowly when females were more susceptible to the *Bt* toxin than males ($z > 0$).
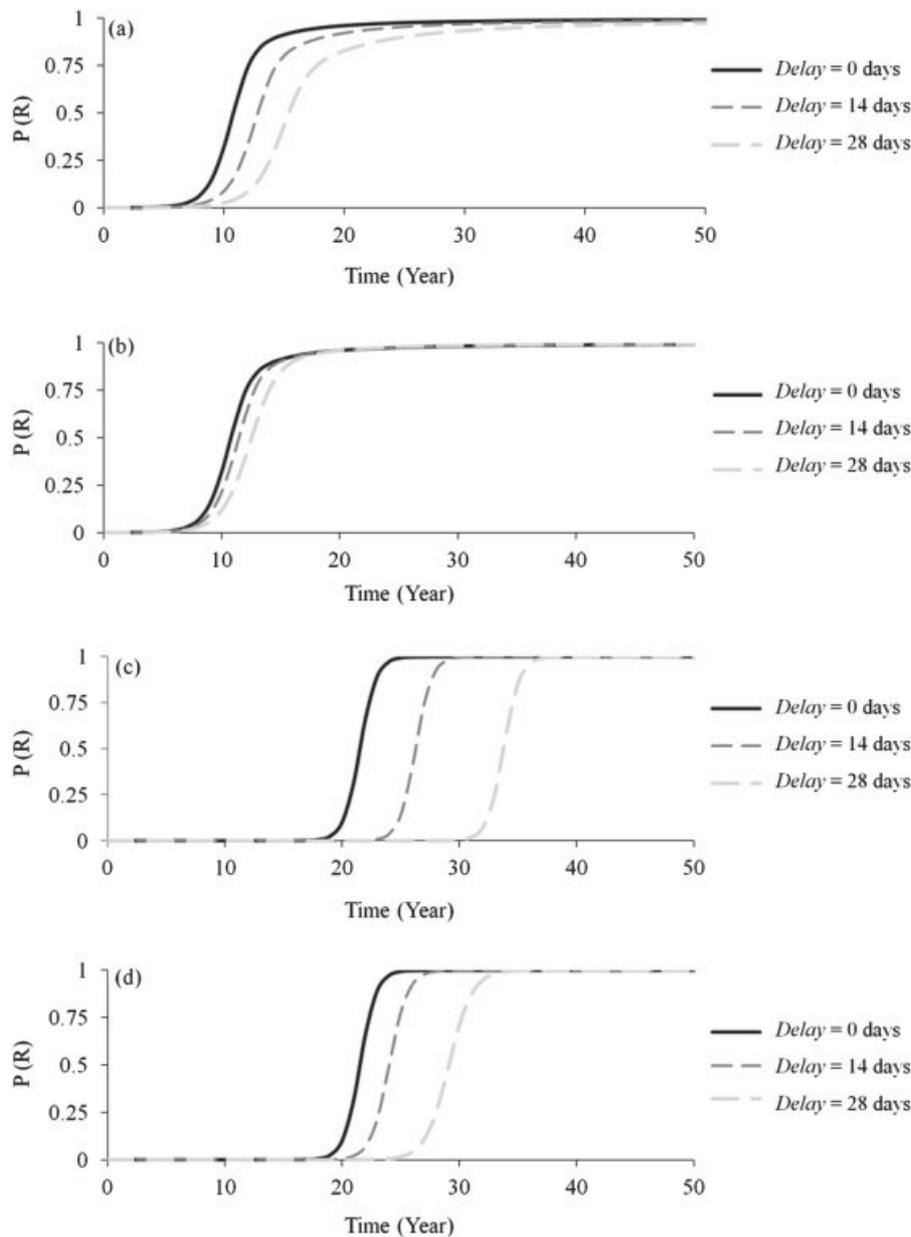
Figure 8. The effect of a time shift in the emergence for male and female beetles from *Bt* corn on resistance allele frequencies in the population [P(R)]. (a) The effect of a time shift in the emergence for male beetles of all genotypes from single-toxin *Bt* corn. (b) The effect of a time shift in the emergence for female beetles of all genotypes from single-toxin *Bt* corn. (c) The effect of a time shift in the emergence for male beetles of all genotypes from pyramided *Bt* corn. (d) The effect of a time shift in the emergence for female beetles of all genotypes from pyramided *Bt* corn. If *Delay* is greater than 0, there is an emergence delay. There is early emergence if *Delay* is less than 0.

3.4 Maturation delay

The effects of a maturation delay or early emergence were studied with toxin–mortality parameters at their standard settings. The emergence delay for beetles from *Bt* corn reported in the two field studies (i.e. an emergence delay of less than 9 days for beetles in *Bt* corn) did not significantly reduce the durability of single-toxin or pyramided *Bt* corn in the scenarios simulated for this study.[11,13] Every 4 day emergence delay for beetles of all genotypes from *Bt* corn delayed resistance evolution by approximately 1 year (9%) in the case of single-toxin corn (Fig. 6). In the case of pyramided-toxin corn, every 1.8 day emergence delay for beetles of all genotypes from *Bt* corn delayed evolution of *Bt* resistance by approximately 1 year (8%) (Fig. 7). If there was a delay in emergence from *Bt* corn

for homozygous resistant and heterozygous beetles (i.e. R1R1 and R1S1 in the case of single-toxin *Bt* corn and R1R1|R2R2, R1S1|R2R2, R1R1|R2S2, R1S1|R2S2, S1S1|R2S2 and R1S1|S2S2 genotypes in the case of pyramided *Bt* corn), resistance to *Bt* corn evolved more slowly.

Bt resistance evolved more rapidly if the emergence of beetles of the homozygous susceptible genotype from *Bt* corn was delayed. However, the effect of an emergence delay for homozygous susceptible beetles from *Bt* corn on *Bt* resistance evolution was not significant. The durability of single-toxin and pyramided *Bt* corn was not significantly affected by a 1–28 day emergence delay for beetles of the homozygous susceptible genotype from *Bt* corn.

The effect of an emergence delay for male or female beetles from *Bt* corn on resistance evolution is shown in Fig. 8. The evolution of *Bt* resistance was delayed when there was a maturation delay for all genotypes of male or female beetles from *Bt* corn.

4 DISCUSSION

Female beetles of *D. v. virgifera* are capable of laying eggs for over 100 days.[31] Oviposition stops by the time of frost, which is Julian day 287 in the present model.[34] Consequently, earlier emergence can be expected to increase the likelihood that individual females will maximize their reproductive output. For example, if there is an emergence delay for female beetles from *Bt* corn, those females will have less time for oviposition than females from non-*Bt* corn. Therefore, the number of eggs laid by a female beetle emerging from *Bt* corn is likely to be less than the number laid by a female emerging from non-*Bt* corn. If there is an emergence delay for male beetles from *Bt* corn, these late-emerging males will mate with females which will then have less time for oviposition than female beetles mating with male beetles in non-*Bt* corn. This results in decreased fitness of male beetles emerging from *Bt* corn.

The effect of maturation delay for *D. v. virgifera* in *Bt* corn in cornfields with a block refuge or a row strip refuge on resistance evolution was studied by Pan *et al.*[18] and Onstad *et al.*[32] The model of Onstad *et al.*[32] simulated a 3–9 day emergence delay for homozygous susceptible beetles from transgenic corn in the field with row strip or block refuges, and they concluded that an emergence delay by susceptible beetles and the configuration of the refuge significantly accelerated the evolution of resistance by *D. v. virgifera*. The model of Pan *et al.*[18] simulated a 7–14 day emergence delay for heterozygous and homozygous susceptible beetles from *Bt* corn in the field with a seed blend or block refuge. They assumed that (1) heterozygous beetles emerged similarly to homozygous susceptible beetles, 2 homozygous susceptible and heterozygous female beetles in *Bt* corn emerged 7 days after male beetles in *Bt* corn and (3) homozygous resistant beetles in *Bt* corn emerged at the same time as all genotypes in non-*Bt* corn. They showed that this type of maturation delay slightly delayed the evolution of *Bt* resistance by *D. v. virgifera*. The present study supports Pan *et al.*[18]

The present study is not without some limitations. Firstly, the model simulated the effect of temporal asynchrony of beetle emergence from *Bt* corn and non-*Bt* corn on resistance evolution, without considering spatial separation of beetles emerging from *Bt* and non-*Bt* corn, which is affected by refuge configuration. Secondly, the physiological changes that may be caused by an extended maturation period in these insects were not modeled. Some physiological changes may affect the mating dynamics of beetles emerging from *Bt* and non-*Bt* corn. The proportion of mating males was assumed to be affected only by age for this model. However, an extended maturation period may affect male competition.[39] Low mating ability/opportunities of late-emerging male beetles or low fecundity of late-emerging female beetles may be consequences of an extended maturation period. Thirdly, sublethal effects of *Bt* toxins, excluding a maturation delay, were not modeled for this study. This model assumed that *Bt* toxins affect only survival rate and maturation. The various and undocumented potential sublethal effects of *Bt* toxins should be studied under field conditions in the future to improve IRM modeling for *D. v. virgifera*.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Levine E and Oloumi-Sadeghi H, Management of diabroticite rootworms in corn. *Annu Rev Entomol* **36**:229–255 (1991).
2 Ball HJ and Weekman GT, Insecticide resistance in the adult western corn rootworm in Nebraska. *J Econ Entomol* **55**:439–441 (1962).
3 Metcalf RL, Implications and prognosis of resistance to insecticides, in *Pest Resistance to Pesticides*, ed. by Georghiou PGP and Sato T. Plenum Press, New York, NY, pp. 703–733 (1983).
4 Meinke LJ, Siegfried BD, Wright RJ and Chandler LD, Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. *J Econ Entomol* **91**:594–600 (1998).
5 Gray ME, Sappington TW, Miller NJ, Moeser J and Bohn MO, Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. *Annu Rev Entomol* **54**:303–321 (2009).
6 Onstad DW, Mitchell PD, Hurley TM, Lundgren JG, Porter PR, Krupke CH *et al.*, Seeds of change: corn seed mixtures for resistance management and integrated pest management. *J Econ Entomol* **104**:343–352 (2011).
7 Gassmann AJ, Petzold-Maxwell JL, Keweshan RS and Dunbar MW, Field-evolved resistance to *Bt* maize by western corn rootworm. *PLoS ONE* **6**(7):e22629 (2011).
8 Onstad DW, *Insect Resistance Management: Biology, Economics, and Prediction*. Academic Press, San Diego, CA, p. 305 (2008).
9 Spencer JL, Isard SA and Levine E, Movement of western corn rootworm adults within and between fields: implications for resistance management. *Proc Illinois Crop Protection Technology Conf*, University of Illinois Extension, Urbana-Champaign, IL, 5–6 January, pp. 28–34 (2005).
10 Storer NP, A spatially explicit model simulating western corn rootworm (Coleoptera: Chrysomelidae) adaptation to insect-resistant maize. *J Econ Entomol* **96**:1530–1547 (2003).
11 Hibbard BE, Frank DL, Kurt R, Boudreau E, Ellersieck MR and Odhiambo JF, Mortality impact of *Bt* transgenic maize roots expressing eCry3.1Ab, mCry3A, and eCry3.1Ab plus mCry3A on western corn rootworm larvae in the field. *J Econ Entomol* **104**:1584–1591 (2011).
12 Storer NP, Babcock JM and Edwards JM, Field measures of western corn rootworm (Coleoptera: Chrysomelidae) mortality caused by Cry34/Cry35b1 proteins expressed in maize event 59122 and implications for trait durability. *J Econ Entomol* **99**:1381–1387 (2006).
13 Murphy AF, Ginzel MD and Krupke CH, Evaluating western corn rootworm (Coleoptera: Chrysomelidae) emergence and root damage in a seed mix refuge. *J Econ Entomol* **103**:147–157 (2010).
14 Al-Deeb MA and Wilde GE, Effect of *Bt* corn expressing the Cry3Bb1 toxin on western corn rootworm (Coleoptera: Chrysomelidae) biology. *J Kansas Entomol Soc* **78**:142–152 (2005).
15 Oyediran IO, Hibbard BE and Clark TL, Western corn rootworm (Coleoptera: Chrysomelidae) beetle emergence from weedy Cry3Bb1 rootworm-resistant transgenic corn. *J Econ Entomol* **98**:1679–1684 (2005).
16 Oyediran IO, Higdon ML, Clark TL and Hibbard BE, Interactions of alternate hosts, postemergence grass control, and rootworm-resistant transgenic corn on western corn rootworm (Coleoptera: Chrysomelidae) damage and adult emergence. *J Econ Entomol* **100**:557–565 (2007).
17 Meinke LJ, Sappington TW, Onstad DW, Guillemaude T, Miller NJ, Komáromi J *et al.*, Western corn rootworm (*Diabrotica virgifera virgifera* Le Conte) population dynamics. *Agric For Entomol* **11**:29–46 (2009).
18 Pan ZQ, Onstad DW, Nowatzki TM, Stanley BH, Meinke LJ and Flexner LJ, Western corn rootworm (Coleoptera: Chrysomelidae) dispersal and adaptation to single-toxin transgenic corn deployed with block or blended refuge. *Environ Entomol* **40**:964–978 (2011).
19 Binning RR, Lefko SA, Millsap AY, Thompson SD and Nowatzki TM, Estimating western corn rootworm (Coleoptera: Chrysomelidae) larval susceptibility to event DAS-59122-7 maize. *J Appl Entomol* **134**:551–561 (2010).
20 Onstad DW, Modeling larval survival and movement to evaluate seed mixtures of transgenic corn for control of western corn rootworm (Coleoptera: Chrysomelidae) *J Econ Entomol* **99**:1407–1414 (2006).

21 Carroll MW, Head G and Caprio M, When and where a seed mix refuge makes sense for managing insect resistance to *Bt* plants. *Crop Prot* **38**:74–79.

22 Devos Y, Meihls LN, Kiss J and Hibbard BE, Resistance evolution to the first generation of genetically modified *Diabrotica*-active *Bt* maize events by western corn rootworm: management and monitoring considerations. *Transgen Res* **22**:269–299 (2013).

23 Hibbard BE, Meihls LN, Ellersick MR and Onstad DW, Density-dependent and density-independent mortality of the western corn rootworm: impact on dose calculations of rootworm-resistant *Bt* corn. *J Econ Entomol* **103**:77–84 (2010).

24 Onstad DW and Meinke LJ, Modeling evolution of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to transgenic corn with two insecticidal traits. *J Econ Entomol* **103**:849–860 (2010).

25 Sutter GR, Branson TF, Fisher JR and Elliott NC, Effect of insecticides on survival, development, fecundity, and sex ratio in controlled infestation of western corn rootworm (Coleoptera: Chrysomelidae). *J Econ Entomol* **84**:1905–1912 (1991).

26 Cates MD, Behavioral and physiological aspect of mating and oviposition by the adult western corn rootworm, *Diabrotica virgifera* Leconte. *PhD Thesis*, University of Nebraska, Lincoln, NE (1968).

27 Marquardt PT and Krupke CK, Dispersal and mating behavior of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in *Bt* cornfields. *Environ Entomol* **38**:176–182 (2009).

28 Quiring DT and Timmins PR, Influence of reproductive ecology on feasibility of mass trapping *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *J Appl Ecol* **27**:965–982 (1990).

29 Lew AC and Ball HJ, Effect of copulation time on spermatozoan transfer of *Diabrotica virgifera* (Coleoptera: Chrysomelidae). *Ann Entomol Soc Am* **73**:360–361 (1980).

30 Kang J and Krupke CH, Likelihood of multiple mating in *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *J Econ Entomol* **102**:2096–2100 (2009).

31 Branson TF and Johnson RD, Adult western corn rootworms: oviposition, fecundity, and longevity in the laboratory. *J Econ Entomol* **66**:417–418 (1973).

32 Onstad DW, Guse CA, Spencer JL, Levine E and Gray ME, Modeling the dynamics of adaptation to transgenic corn by western corn rootworm (Coleoptera: Chrysomelidae). *J Econ Entomol* **94**:529–540 (2001).

33 Boetel MA and Fuller BW, Seasonal emergence time effect on adult longevity, fecundity, and egg viability of northern and western corn rootworm (Coleoptera: Chrysomelidae). *Environ Entomol* **26**:1208–1212 (1997).

34 Ball HJ, On the biology and egg-laying habits of the western corn rootworm. *J Econ Entomol* **50**:126–128 (1957).

35 Godfrey LD, Meinke LJ, Wright RJ and Hein GL, Environmental and edaphic effects on western corn rootworm (Coleoptera: Chrysomelidae) overwintering egg survival. *J Econ Entomol* **88**:1445–1454 (1995).

36 Gray ME, Survey of Illinois cornfield reveals exceedingly low densities of western corn rootworm adults. Bulletin No. 18, University of Illinois Extension, Article 1 (2011).

37 Gray ME, Estes RE and Tinsley N, Insect surveys in producers' corn and soybean fields: what did we learn? *Proc University of Illinois Corn and Soybean Classic*, University of Illinois, Champaign-Urbana, *IL*, pp. 22–30 (2012).

38 Godfrey LD and Turpin FT, Comparison of western corn rootworm (Coleoptera: Chrysomelidae) adult populations and economic thresholds in first-year and continuous cornfields. *J Econ Entomol* **76**:1028–1032 (1983).

39 Spencer J, Onstad D, Krupke C, Hughson S, Pan Z, Stanley B *et al.*, Isolated females and limited males: evolution of insect resistance in structured landscapes. *Entomol Exp Appl* **146**:38–49 (2013).