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Modeling Evolution of Resistance by *Maruca vitrata* (Lepidoptera: Crambidae) to Transgenic Insecticidal Cowpea in Africa

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ABSTRACT We created a detailed model of the *Maruca vitrata* (F.) and cowpea [*Vigna unguiculata* (L.) Walp] system to study the possible evolution of resistance by the insect to transgenic insecticidal cowpea, which is under development. We focused on population dynamics and genetics in a region of west Africa. We simulated single-toxin and pyramided (two-toxin) cowpea and emphasized conservative, worst-case scenarios in our analysis. The results indicate that as long as a pyramided, transgenic cowpea can be developed, seed saving by farmers and reliance on natural refuge are not major problems for resistance management. Furthermore, it is possible that one or both toxins in the pyramid may not need to be high dose for evolution to be delayed significantly (>20 yr or 80 generations for resistance to become a concern if transgenic cowpea is deployed in areas where *M. vitrata* is endemic). If efforts are made to deploy transgenic cowpea only into the regions where *M. vitrata* is not endemic, then there is little to no concern with resistance emerging in the *M. vitrata* population.

KEY WORDS Bt cowpea, resistance management, simulation

Cowpea [*Vigna unguiculata* (L.) Walp] is an important crop in the tropics, and because of its high protein content, constitutes a major staple food for people in sub-Saharan Africa (Langyintuo et al. 2003, Murdock et al. 2008). The main producers in Africa are Nigeria, Niger, and Burkina Faso (FAOSTAT 2009). However, the average yield for a pure crop is 794 kg/ha in Burkina Faso (DGPER/MAHRH 2010), 200 kg/ha in Niger, and 700 kg/ha in Nigeria (computed data from FAOSTAT 2009). These are considerably lower than the estimated potential of 2 tons/ha (Singh et al. 1997). This yield gap is because of several abiotic and biotic constraints. Field and storage insect pests are among the most severe biotic constraints for cowpea production (Singh and Van Emden 1979, Singh et al. 1990).

The bean pod borer *Maruca vitrata* (F.) (Lepidoptera: Crambidae) is one of the most serious pests of cowpea in moist savannas (Taylor 1967). Eggs are deposited randomly on the vegetative buds, flowers, and sometimes on axils of leaves (Taylor 1967; Firempong and Mangalit 1990). Early-instar larvae feed mainly on flowers (Karel 1985). Each larva can consume four to six flowers (Gblagada 1982). Flower infestation rates of up to 80% were reported in west Africa (Afun et al. 1991). Third- to fifth-instars are capable of boring into the pods, and occasionally into peduncle and stems (Taylor 1967). *Maruca vitrata* infestations may reduce yield by 20–80% (Singh et al. 1990). The total life cycle ranges from 22 to 25 d (Singh and Jackai 1988). *Maruca vitrata* does not undergo diapause and the populations of the insect during the off season are maintained on a wide range of host plants (Okeyo-Owuor and Ochieng 1981, Arodokoun et al. 2003). Depending on the agroecological zone, three to four generations of *M. vitrata* occur annually on cowpea and the population survives the dry season on alternative host plants. In west Africa, the importance of alternative hosts varies among the regions, along a south–north gradient. In moister areas where host plants are abundant, *M. vitrata* is present throughout the year. In dryer areas such as Sudan Savanna and Sahelian Savanna, where the dry season lasts 7–8 mo and host plants are rare, *M. vitrata* is perceived by farmers to act like a migratory pest invading cowpea fields during the rainy season, supposedly moving north from the humid areas in the South but also dispersing from local wild hosts (Bottenberg et al. 1997; Ba et al. 2009; Margam et al. 2011a, b).

Although chemical control with synthetic insecticides can be used in west Africa for cowpea pests, this approach is not the optimum tactic in west African

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cowpea production for four reasons: 1) *M. vitrata* populations have evolved resistance to some synthetic insecticides, such as cypermethrin, dimethoate, and endosulfan (Ekesi 1999); 2) a majority of farmers cannot afford chemical insecticides (Bottenberg 1995); 3) natural enemies are often harmed by available insecticides; and 4) many farmers do not have access to proper safety equipment, and many of the farmers have low literacy. Thus, if they do have access to pesticides and safety equipment, they face significant challenges with understanding the instructions required for effective use and safe handling of these compounds.

A potential advance in the management of *M. vitrata* has been achieved by engineering cowpea encoding genes that express the Cry1Ab delta endotoxin of *Bacillus thuringiensis* (Bt) subsp. *kurstaki* (Popelka et al. 2006, Chaudhury et al. 2007, Adesoye et al. 2008, Huesing et al. 2011). (The Cry1Ab technology is registered by Monsanto [Creve Coeur, MO] and was given to the African Agricultural Technology Foundation for use in cowpea in Africa.) However, the sustainability of such a new technology relies on effective insect resistance management (IRM). The purpose of an IRM plan is to significantly delay the evolution of resistance to the Bt toxin in *M. vitrata* populations (Kennedy 2008, Onstad 2008).

Computer simulations are a powerful tool to predict the likelihood of a pest to evolve resistance to insecticidal toxins. To date, such predictions have been the basis for managing resistance to transgenic insecticidal crops and sustainable pest management (Onstad and Gould 1998; Medvinskya et al. 2004; Onstad 2008). So far most of the cases of IRM modeling pertain to transgenic crops in developed countries (Onstad 2008). The context is more complex in developing countries, where deployment of IRM plans in rural areas is difficult. For example, one challenge is providing educational materials to a mostly illiterate farm population consisting of diverse language and cultural groups. These difficulties make it nearly impossible to enforce IRM plans. Also for cowpea in Africa, we must consider that small-scale farmers typically own <1 ha of land (Ogungbile et al. 1998), farmers save seed from 1 yr to plant in the next year, and they share seed. Once Bt cowpea is released, regulatory agencies will have limited ability to prevent the spread and use of Bt cowpea seeds across regions where it may not be recommended (because of IRM-related issues) to be grown. However, efforts can be made to promote the use of Bt cowpea in regions or scenarios where problems with resistance can be minimized. Finally, although the majority of cowpea production occurs in regions where *M. vitrata* is not endemic, regions exist where cowpea is grown and *M. vitrata* occurs throughout the year (Kossou et al. 2001, Arodokoun et al. 2003, Ba et al. 2009). In addition, we cannot rule out the possibility that introduction of Bt cowpea into west Africa may result in increased production in those areas where *M. vitrata* is endemic, because of the fact that the Bt toxin will help to reduce insect pressure from one pest species on cowpea crops. As this endemic region is thought to be the source population for *M. vitrata* that migrate into the major cowpea production areas, it is critical to understand the potential for resistance in the *M. vitrata* population if Bt cowpea is grown in the endemic zone.

The goal of this study is to determine, before Bt cowpea development is finished, the crop and landscape conditions that will enable sustainable use of Bt cowpea. Sustainable use of a transgenic insecticidal crop depends on maintaining efficacy of the crop for the most farmers over the longest period of time. We believe that a technology that lasts 15–20 yr is worth developing and implementing. In the case of *M. vitrata*, if Bt cowpea is deployed in the zone where *M. vitrata* is endemic, this durability would provide cowpea protection for 60–80 generations. In addition, recommended deployment strategies (i.e., deployment of Bt cowpea only in nonendemic zones) that could extend the lifetime of this technology far beyond 20 yr would be highly beneficial. Specific objectives include 1) determining how low the mortality caused by the Bt cowpea can be; 2) determining if natural refuge and traditional cowpea crops provide enough refuge to delay resistance; and 3) based on these aforementioned questions, where should researchers focus their attention in future work?

Materials and Methods

Population Genetics. We used a simple population genetics model representing several insect populations in a landscape of wild host plants and cowpea. Some of the cowpea crops do not express insecticidal traits, some express one toxin, and some express two independent insecticidal traits. Two autosomal, diallelic, resistance genes are modeled in the insect population: the first locus has one major gene designated with X for wild type and Y for resistance to plant trait 1, and the second locus has another major gene designated with S for wild type and R for resistance to plant trait 2. We assumed that the two genes are independent of each other and that mutations do not occur after the start of the simulations.

Model Landscape. The model consists of three hierarchical levels of hypothetical space. At the largest scale, the model represents three geographical regions (Fig. 1). The southern region (region 1) maintains the population of the pest all year and acts as a source for immigrants into the other two regions. The other two regions receive migrants once per year. The second or middle region has fewer wild hosts and thus is not a source for migration into other regions, but it has more and better wild habitat than the third (northern) region. This simplification of reality is a first step to model some areas of west Africa (Langyintuo et al. 2003). In the simplest version of the model, we define only one region (omitting migration).

Each region consists of two kinds of fields of host plants representing the typical area infested by *M. vitrata.* By field we mean either a cowpea field or an area occupied by wild host plants. In the model, cowpea fields consist of 7–19 types of cowpea plants in

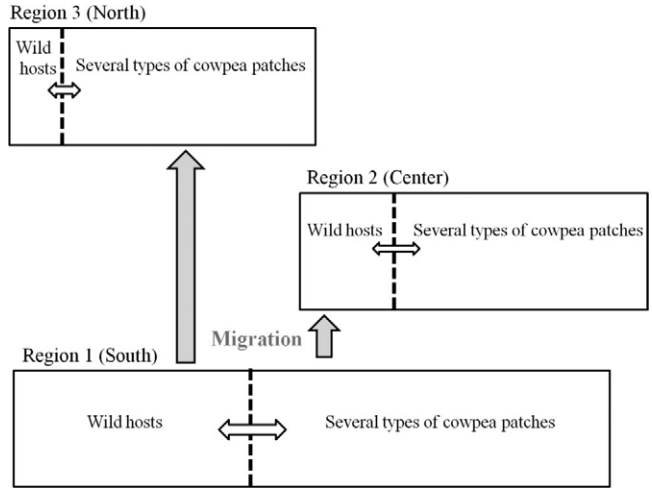

Fig. 1. Spatial design of model for *M. vitrata* in west Africa.

various dynamic proportions. With single-toxin cowpea, we defined seven plant types (Table 1). For scenarios with pyramided Bt cowpea, 19 plant types are based on the combinations produced with two plant loci each expressing three levels of toxin: none, intermediate, or full (Table 2). Thus, a refuge plant is represented by two loci with no expression of toxin, whereas a standard pyramided Bt cowpea plant has two fully expressing loci. We expect that a cowpea field with a mixture of seeds expressing 0, 1, or 2 toxins will be common if farmers collect their own seed for later use and mix seed collected from multiple fields. Other mixtures of fully-expressing and partially-expressing plants may also exist after cross-pollination occurs. Such fields will be common if nontransgenic plants are grown close to blocks of certified seed. The seeds resulting from cross-pollination may not fully express the toxin(s). In the model, cowpea is primarily a self-pollinated crop producing viable pollen and receptive stigma before anthesis. However, during each generation, outcrossing may occur in 1% of the flowers (Fatokun and Ng 2007, Asiwe 2009) because of movement of pollen by bees (Pasquet et al. 2008, Asiwe 2009, Fohouo et al. 2009).

The smallest spatial scale in the model is the patch. A patch represents the proportion of each of the plant types in each cowpea field. One cowpea crop is planted each year, but planting times are asynchronous across each region. Tables 1 and 2 display the matrices that describe how the patches annually change in the cowpea areas of each region. We assume that each toxin is based on the expression of one gene. Because intermediate levels of toxicity may contribute to differential selection between homozygous susceptible and heterozygous insects even when both are susceptible at higher toxin doses (Onstad 2008), we take a conservative approach and permit cross-pollination to create cowpea plants of intermediate toxicity (Tables 1 and 2). Our approach can be thought of as simulating one or two homozygous plant genes in purchased Bt cowpea (Huesing et al. 2011) and nontoxic homozygous

Table 1. New proportional areas, P, of patches defined by single-toxin expression in cowpea

Period	Patch	Toxicity[a]	Equation based on P for previous year, y − 1
y = 1	1	0	1.0 − Tr
	7[b]	1	Tr
y > 1	1	0	P(1,c) = (1 − Q) × [(1 − (Cr × 0.5))× P(1,c) + CR × 0.5 × P(2,c)]
	2	0.5	P(2,c) = (1 − Q) × {(1 − Cr) × P(2,c) + Cr × [0.5 × P(1,c) + 0.5 × P(3,c)]}
	3	1	P(3,c) = (1 − Q) × {(1 − (Cr × 0.5)) × P(3,c) + Cr × 0.5 × P(2,c)}
	4[c]	0	P(4,c) = (1 − Q) × [(1 − Cr) × P(4,c) + CR × 0.5 × P(5,c)]
	5[c]	0.5	P(5,c) = (1 − Q) × {(1 − Cr) × P(5,c) + Cr × [P(4,c) + 0.5 × P(6,c) + 0.5 × P(7,c)]}
	6[c]	1	P(6,c) = (1 − Q) × {(1 − (Cr × 0.5)) × [P(6,c) + P(7,c)] + Cr × 0.5 × P(5,c)}
	7[b]	1	P(7,c) = y × Tr 1< y ≤ 5
			P(7,c) = P(7,c) y > 5

Values of P on left side are for current year; values on right side are for previous year. Q is the proportion of land planted with new Bt cowpea in year y.

Standard Tr = 0.1 (proportion of transgenic cowpea in year 1); standard cross-pollination, Cr = 0.01.

[a] The number from 0 to 1 represents the relative toxicity of the toxin. Zero means no expression of toxin and RT = 0.5 means intermediate expression.

[b] Patch 7 is a block of pure Bt cowpea plants; the other patches are seed mixtures.

[c] Patches 4–6 represent separately saved batch of seed (from purchased seed) and are planted separately from patches 1–3 (traditional seed).

Table 2. New proportional areas, P, of cowpea patches affected by pyramided Bt cowpea

Period	Patch	Toxicity[a]	Equation based on P for previous year, y − 1
y = 1	1	0, 0	1.0 − Tr
	19[b]	1, 1	Tr
y > 1	1	0, 0	$P(1,c) = (1 - Q) \times [(1 - Cr \times 0.5) \times P(1,c) + CR \times 0.25 \times [P(2,c) + P(3,c)]$
	2	0, 0.5	$P(2,c) = (1 - Q) \times \{(1 - Cr \times 0.75) \times P(2,c) + Cr \times 0.25 \times [P(1,c) + P(6,c) + P(4,c)]\}$
	3	0.5, 0	$P(3,c) = (1 - Q) \times \{(1 - Cr \times 0.75) \times P(3,c) + Cr \times 0.25 \times [P(1,c) + P(6,c) + P(5,c)]\}$
	4	0, 1	$P(4,c) = (1 - Q) \times \{(1 - Cr \times 0.5) \times P(4,c) + Cr \times 0.25 \times [P(7,c) + P(2,c)]\}$
	5	1, 0	$P(5,c) = (1 - Q) \times \{(1 - Cr \times 0.5) \times P(5,c) + Cr \times 0.25 \times [P(8,c) + P(3,c)]\}$
	6	0.5, 0.5	$P(6,c) = (1 - Q) \times \{(1 - Cr) \times P(6,c) + Cr \times 0.25 \times [P(7,c) + P(8,c) + P(2,c) + P(3,c)]\}$
	7	0.5, 1	$P(7,c) = (1 - Q) \times \{(1 - Cr \times 0.75) \times P(7,c) + Cr \times 0.25 \times [P(9,c) + P(6,c) + P(4,c)]\}$
	8	1, 0.5	$P(8,c) = (1 - Q) \times \{(1 - Cr \times 0.75) \times P(8,c) + Cr \times 0.25 \times [P(9,c) + P(6,c) + P(5,c)]\}$
	9	1, 1	$P(9,c) = (1 - Q) \times \{(1 - Cr \times 0.5) \times P(9,c) + Cr \times 0.25 \times [P(7,c) + P(8,c)]\}$
	10[c]	0, 0	$P(10,c) = (1 - Q) \times [(1 - Cr \times 0.5) \times P(10,c) + CR \times 0.25 \times [P(11,c) + P(12,c)]$
	11[c]	0, 0.5	$P(11,c) = (1 - Q) \times \{(1 - Cr \times 0.75) \times P(11,c) + Cr \times 0.25 \times [P(10,c) + P(15,c) + P(13,c)]\}$
	12[c]	0.5, 0	$P(12,c) = (1 - Q) \times \{(1 - Cr \times 0.75) \times P(12,c) + Cr \times 0.25 \times [P(10,c) + P(15,c) + P(14,c)]\}$
	13[c]	0, 1	$P(13,c) = (1 - Q) \times \{(1 - Cr \times 0.5) \times P(13,c) + Cr \times 0.25 \times [P(16,c) + P(11,c)]\}$
	14[c]	1, 0	$P(14,c) = (1 - Q) \times \{(1 - Cr \times 0.5) \times P(14,c) + Cr \times 0.25 \times [P(17,c) + P(12,c)]\}$
	15[c]	0.5, 0.5	$P(15,c) = (1 - Q) \times \{(1 - Cr) \times P(15,c) + Cr \times 0.25 \times [P(16,c) + P(17,c) + P(11,c) + P(12,c)]\}$
	16[c]	0.5, 1	$P(16,c) = (1 - Q) \times \{(1 - Cr \times 0.75) \times P(16,c) + Cr \times 0.25 \times [P(18,c) + P(19,c) + P(15,c) + P(13,c)]\}$
	17[c]	1, 0.5	$P(17,c) = (1 - Q) \times \{(1 - Cr \times 0.75) \times P(17,c) + Cr \times 0.25 \times [P(18,c) + P(19,c) + P(15,c) + P(14,c)]\}$
	18[c]	1, 1	$P(18,c) = (1 - Q) \times \{(1 - Cr \times 0.5) \times [P(18,c) + P(19,c)] + Cr \times 0.25 \times [P(16,c) + P(17,c)]\}$
	19[b]	1, 1	$P(19,c) = y \times Tr \quad 1 < y \leq 5$
			$P(19,c) = P(19,c) \quad y > 5$

Values of P on left side are for current year; values on right side are for previous year. Q is the proportion of land planted with new Bt cowpea in year y. Standard Tr = 0.1 (proportion of transgenic cowpea in year 1); standard cross-pollination, Cr = 0.01.

[a] The number from 0 to 1 represents the relative toxicity of each of two toxins that can possibly be expressed in each plant. A refuge plant (0,0) has no expression of toxins. A pyramid of two toxins has 1, 1. The RT = 0.5 represents intermediate expression.

[b] Patch 19 is a block of pure Bt cowpea plants; the other patches are seed mixtures.

[c] Patches 10–18 represent separately saved batch of seed (from purchased seed) and are planted separately from patches 1–9 (traditional seed).

genes in traditional cowpea. Plants with intermediate toxicity are hemizygous for at least one trait.

We assume that each year new Bt cowpea seed is purchased from government-sanctioned businesses or obtained from a nongovernmental organization or government agency. To account for the worst case scenario, we model farmer saving of transgenic seed for planting the next year. We assume that adoption of Bt cowpea will increase over time and that half of the cowpea fields will be planted with Bt cowpea by year 5.

We assume that for all saved seed, 1% of the seed has been contaminated by pollen from refuge cowpea or certified Bt cowpea in equal proportions. This may occur similarly by the process described by Chilcutt and Tabashnik (2004) and Heuberger et al. (2008a, b) for transgenic insecticidal crops and associated refuge. We also assume that cross-pollination does not change conditions during current year. This simplification means that pollination of refuge plant flowers that produces insecticidal seed will not be toxic in the current year. In addition, pollination of toxic flowers produces refuge seed but the larvae will have to eat toxic flower and pod tissue before consuming seeds.

Generations of *M. vitrata*. Generations are discrete, meaning that individuals of one generation cannot mate with offspring of that generation. We define generation 1 as the first generation of larvae on wild hosts after the end of cowpea production. In the standard model there is a total of nine generations: five occur only on wild hosts and four occur on both types of hosts. For simplicity, we assume that the insect generations are synchronized across all regions.

Although climatic cycles in west Africa cause cycling of pest population abundances, we will not be modeling this aspect of the system. No evidence of diapause in *M. vitrata* has been found (Taro Adati, Department of International Agricultural Development, Tokyo University of Agriculture, personal communication). We argue that management of resistance does not need to be based on the modeling of realistic weather patterns, unless these patterns impose stresses on only one phenotype and not others.

Submodel for *M. vitrata* on Cowpea

Density and Dispersal of Adults. Adults invade cowpea at the end of generation 5, are produced in generations 6–9, but leave cowpea at end of generation 9 and move to wild hosts. The densities of males and females emerging in each region, c, are calculated either from the density of adults on wild hosts, w, $A_{i,w,c}$ (t), or from the density of older larvae, $ML_{i,p,c}$ (t).

$$\begin{aligned} F_{i,p,c}(5) &= 0.5 \times P(p,c) \times DWC \times A_{i,w,c}(5) \\ F_{i,p,c}(t) &= 0.5 \times ML_{i,p,c}(t) \quad \text{for } t = 6 \text{ to } 8 \\ F_{i,p,c}(9) &= 0 \end{aligned} \qquad [1]$$

$$\begin{aligned} M_{i,c}(5) &= 0.5 \times DWC \times Ai,w,c(5) \\ M_{i,c}(t) &= 0.5 \times \sum_{p=1}^{Ptot} ML_{i,p,c}(t) \quad \text{for } t = 6 \text{ to } 8 \\ M_{i,c}(9) &= 0 \end{aligned} \qquad [2]$$

where F and M are the densities of female and males in genotype i, P is the proportional area of patch p, Ptot is the total number of patches (Tables 1 and 2), and DWC is the proportion dispersing from wild hosts to cowpea. Our standard value for DWC is 0.5. Half of the population is female (Atachi and Gnanvossou 1989, Lu et al. 2007). See equation 14 for descriptions of mated females in generation five based on migration.

Maruca vitrata occurs in a landscape of cowpea crops and wild hosts. We assume that in a given region, these are all found near each other so that dispersal among plant types can occur in each generation of *M. vitrata.* The pest has evolved the ability to move every generation or two to a new host plant that is flowering. Thus, we believe that the pest has evolved good foraging and searching abilities. For simplicity, we also assume that *M. vitrata* has no preference for one plant species over another. The adult preference for flowering plants and the asynchrony in maturation across crop fields and between wild and cultivated species contributes to the mixing of *M. vitrata* populations. We assume that adults in cowpea patches randomly mate but cannot mate with those in wild hosts, and vice versa. Adults are nocturnal (Lu et al. 2007) and the highest mating frequency occurs in 3-d-old females (Huang and Peng 2001, Lu et al. 2007). Females mate only once (Atachi and Gnanvossou 1989, Jackai et al. 1990).

Oviposition. The mean fecundity per female emerging in nontransgenic cowpea is 400 eggs (Jackai et al. 1990). We assume that all eggs are viable and that feeding and surviving on Bt cowpea does not reduce fecundity in female moths. The mated females uniformly distribute the eggs across the cowpea fields and patches within each major region. The number of eggs $E_{i,p,c}\ (t+1)$ of genotype i in patch p in region c for generation t + 1 as a function of the number of female moths in generation t is

$$E_{i,p,c}(t+1) = P(p,c) \times \sum_{g=1}^{9} \sum_{n=1}^{Ptot} \sum_{m=1}^{9} [b_{g,n,c} \times FM_{g,n,m,c}\ (t) \times w_{i,g,m}]$$

$$FM_{g,n,m,c}\ (t) = F_{g,n,c}\ (t) \times Z_{m,c}\ (t) \qquad [3]$$

where P(p,c) is the proportion of each type of plant in the cowpea field and b is the fecundity based on female genotype and natal patch. F is the density of females in genotype g emerging from patch n out of a total Ptot; FM is the number of mated females, and Z is the frequency of mates that are genotype m in the region. Each weight, w, equals the Mendelian proportion of all offspring that are genotype i when genotypes g and m mate. A constant b = 400 is used in all simulations.

Equations for Larvae. We modeled the average larva in a genotype in each patch. Except for the case in which Bt cowpea seed is purchased and planted in a separate block, we consider every cowpea field to be a seed mixture. Larvae on wild hosts also are modeled separately.

Table 3. Probability of survival due to toxicity. Parameters h1 and h2 indicate the level of dominance for the two major resistance alleles Y and R, respectively

Two major genes	Survival
XXSS	Smin1 × Smin2
XYSS	[h1 × Smax1 + (1 − h1) × Smin1] × Smin2
YYSS	Smax1 × Smin2
XXSR	Smin1 × [h2 × Smax2 + (1 − h2) × Smin2]
XYSR	[h1 × Smax1 + (1 − h1) × Smin1] × [h2 × Smax2 + (1 − h2) × Smin2]
YYSR	Smax1 × [h2 × Smax2 + (1 − h2) × Smin2]
XXRR	Smin1 × Smax2
XYRR	[h1 × Smax1 + (1 − h1) × Smin1] × Smax2
YYRR	Smax1 × Smax2

All six parameters in the algorithm are specific for each patch type (not shown). Smin1 and Smin2 are survival for the susceptible homozygotes XX and SS, respectively. Smax1 and Smax2 are the max survival provided by each of the two major resistance alleles Y and R, respectively.

Toxin Mortality. In the model, density-independent toxin mortality incurred by larvae depends upon genotype and the dominance of the resistance allele. We assumed this mortality (1−Stox) is applied to neonates feeding on cowpea flowers before movement and competition-based survival. The two genes have independent, multiplicative effects on resistance. For patch 1 (the refuge cowpea plants), no mortality because of toxin occurs and the Stox values are all 1. Table 3 presents the survival rates (Stox) for each genotype not settling on refuge plants in a patch described by Tables 1 and 2. The algorithm in Table 3 depends on six parameters. The h values are dominance of resistance. Smin1 and Smin2 are the survival rates because of plant traits 1 and 2. Smax1 and Smax2 are the maximum survival rates by resistant homozygotes. Each insect locus affects survival in heterozygotes according to the formula: h1 × Smax1 + (1−h1) × Smin1. Total survival Stox cannot exceed 1.

The values of the six parameters are dependent on which patch the pest infests. The proportional reduction in toxin mortality because of plant patch is RT1 or RT2 (relative toxicities in Tables 1 and 2). A value of RT = 1 means that there is no reduction in mortality in response to a plant trait. These values are used to calculate the values of Smin and Smax.

$$\begin{aligned} Smin1p &= 1 - [(1 - SN1) \times RT1p] \\ Smin2p &= 1 - [(1 - SN2) \times RT2p] \\ Smax1p &= 1 - [(1 - SX1) \times RT1p] \\ Smax2p &= 1 - [(1 - SX2) \times RT2p] \end{aligned} \qquad [4]$$

where SN is the survival of a susceptible homozygote on a full dose of one toxin and SX is the survival of resistant homozygotes on the same kind of plant. The values of RT are also used to calculate the dominance of resistance as toxin exposure and dose change from patch to patch.

$$h1p = (1 - hh1) \times (1 - RT1p) + hh1$$
$$h2p = (1 - hh2) \times (1 - RT2p) + hh2 \quad [5]$$

where hh is the dominance of resistance on pure Bt cowpea plants when RT = 1. The number of third instars, $L1_{i,p,c}(t)$, is calculated according to survival of toxins in patch p.

$$L1_{i,p,c}(t) = [Stox_{i,p,c}]^{d} \times 0.04 \times E_{i,p,c}(t) \quad [6]$$

Our standard assumption is that survival before the third stadium is equal to the square root (d = 0.5) of the total survival observed on Bt cowpea. We assume that weather and other natural density-independent factors kill 96% of first and second instars. In a sensitivity analysis we varied the value of d to change the timing of toxin mortality.

We then calculate survival because of movement from plant-to-plant based on the approach of Tabashnik (1994). We assume that only third instars, L2, move to adjacent plants after feeding on the flowers on which they were oviposited. The first equation represents larvae in the block of pure Bt cowpea [$P = 7$ for single-toxin cowpea (Table 1) or $P = 19$ for pyramided cowpea (Table 2)].

$$L2_{i,p,c}(t) = L1_{i,p,c}(t) \times [Stox_{i,p,c}]^{(1-d)} \quad [7]$$

Note that we assume that there are no off-type seeds in the block of Bt cowpea.

The number settling in patch r in a seed mixture defined in Tables 1 and 2 is

$$L2_{i,r,c}(t) = L1_{i,r,c}(t) \times (1 - V) + P(r,c) \times \sum_{p=jj}^{nn} L1_{i,p,c}(t) \times V \times [Stox_{i,p,c}]^{(1-d)} \quad [8]$$

where V is probability of leaving a plant, P(r,c) is the proportion of the population arriving in cowpea patch r in region c, and $[Stox_{i,p,c}]^{(1-d)}$ is survival before movement as a function of genotype i and patch p. For areas with single-toxin cowpea, the indices jj and nn are either one and three for the patches of saved traditional seed or four and six for the patches of saved cowpea seed saved from plots planted with purchased Bt cowpea (Table 1). For areas with pyramided cowpea, the indices jj and nn are either one and nine for the patches of saved traditional seed or 10 and 18 for the patches of saved cowpea seed saved from plots planted with purchased Bt cowpea (Table 2).

In theory, the probability of leaving a plant V could be a function of the plant type and the larval genotype, but because of the lack of knowledge, we chose to make it a single constant. Furthermore, in none of the equations do we model mortality during larval movement. Refuge is a seed mixture for the standard simulation.

Survival of Older Larvae. We assume that density-dependent mortality occurs after density-independent mortality such as toxin exposure. The total density per ha, TL, of young larvae in genotype i in each kind of patch p in region c is

$$TL_{p,c} = \sum_{i=1}^{9} L2_{i,p,c}(t) \quad [9]$$

We assumed that the larval carrying capacity is 60 per plant and that 60,000 cowpea plants are planted per ha. Survival declines as larval density increases. Then we calculated the number of older larvae, $ML_{i,p,c}(t)$, surviving density-dependent competition,

$$ML_{i,p,c}(t) = L2_{i,p,c}(t) \times \exp\left[-5.3 \times \frac{TL_{p,c}}{(P(p,c) \times 3.6 \times 10^{6}}\right] \quad [10]$$

As TL approaches 3.6 million larvae per ha, the exponential function for survival of larvae approaches 0.005. The value of −5.3 was chosen to produce zero population growth at the carrying capacity based on 400 eggs per female and a 1:1 sex ratio. With one larva per plant, 91.5% survive, which matches the range (80–90%) observed in the field (Jackai and Singh 1983, Adati et al. 2004). Mortality of 1–10% because of natural enemies has been reported in cowpea (Taylor 1967, Arodokoun et al. 2006).

Submodel for *M. vitrata* on Wild Hosts

We assumed that the wild population is maintained for nine generations throughout the year on a variety of wild hosts. The number of adults in genotype i in region c, $A_{i,w,c}$ developing from eggs in wild refuge, w, in generation t is

$$A_{i,w,c}(t) = E_{i,w,c}(t) \quad \text{for all t except 5 and 9.} \quad [11]$$

We assume that moths on wild hosts mate and produce offspring according to Hardy–Weinberg proportions.

The model allows *M. vitrata* to disperse locally before mating (Ba et al. 2009), but we assume that local dispersal and mating occurs in region 1 before migration. In generation 5 before migration, the density on wild hosts in each region is reduced by the proportion dispersing to cowpea, DWC.

$$A_{i,w,c}(5) = (1 - DWC) \times A_{i,w,c}(5) \quad [12]$$

The standard value of DWC is 0.5 for all regions.

At the end of generation 5, mated females, $FM_{i,w,1}(5)$ and $FM_{i,1}(5)$, on wild hosts and cowpea can migrate from region 1 to the other regions.

$$FM_{i,w,1}(5) = (1 - MIG) \times FM_{i,w,1}(5)$$
$$FM_{i,1}(5) = (1 - MIG) \times FM_{i,1}(5)$$
$$FM_{i,2}(5) = 0.6 \times 0.99 \times MIG \times [FM_{i,w,1}(5) + FM_{i,1}(5)]$$
$$FM_{i,w,2}(5) = 0.6 \times 0.01 \times MIG \times [FM_{i,w,1}(5) + FM_{i,1}(5)]$$
$$FM_{i,3}(5) = 0.4 \times 0.999 \times MIG$$

$$\times [FM_{i,w,1}(5) + FM_{i,1}(5)]$$

$$FM_{i,w,3}(5) = 0.4 \times 0.001 \times MIG \times [FM_{i,w,1}(5) + FM_{i,1}(5)] \quad [13]$$

where MIG is the proportion of the region 1 population that migrates out of region and the constants 0.6 and 0.4 represent the fractions that invade regions 2 and 3, respectively. Wild hosts inhabit 1 and 0.1% of regions 2 and 3, respectively, during the cowpea cropping season. We set MIG = 0.36 as our standard value. This means that 36% of the adults in region 1 migrate at end of fifth generation. Sixty-four percent remain on wild hosts in region 1 and continue growing at a small rate of increase.

In generation 9, adults from all patches of cowpea disperse to wild hosts with probability DCW, which differs from region to region.

$$A_{i,w,c}(9) = E_{i,w,c}(9) + DCWc \times \left[M_{i,c}(9) + \sum_{p=1}^{Ptot} F_{i,p,c}(9) \right] \quad [14]$$

The standard values of DCW are 0.5, 0.05 and 0.01 for regions 1, 2, and 3, respectively. These values account for both the availability of wild hosts as well as the different mortality rates experienced during dispersal in each region. Thus, the same proportion that moves to cowpea returns to wild hosts in region 1, but the returning populations are much smaller in the other two regions.

Population Growth. The following equation calculates the number of eggs on wild hosts in region c:

$$E_{i,w,c}(t+1) = 0.5 \times \sum_{g=1}^{9} NRR_{g,c} \times A_{g,w,c}(t) \times \sum_{m=1}^{9} w_{i,g,m} Q_{m,c}(t) \quad [15]$$

where 0.5 is the proportion of females and NRR is the net reproductive rate for phenotype g for *M. vitrata* on wild hosts. A fitness cost for resistant phenotypes is modeled as a lower NRR value relative to susceptible phenotypes. The value of NRR was adjusted in the model to mimic maintenance of *M. vitrata* on wild hosts. We likely need some increase in the wild population in region 1 because the migrants must be produced by and subtracted from the endemic wild population each year. We use $NRR_{g,c} = 2$ for susceptible phenotypes, so that the population density starting on wild hosts remains stable. We know that *M. vitrata* evolved in the wild and therefore must be somewhat fit to persist indefinitely. However, long-term persistence may depend on significant population growth on wild hosts during the four generations occurring during good weather (coincidental with cowpea production).

Survival of *M. vitrata* may be much lower on wild host plants because of natural enemies. The two most important parasitoids (Hymenoptera: Braconidae) were *Phanerotoma leucobasis* Kriechbaumer and *Braunsia kriegeri* Enderlein, which were observed all year on various host plants by Arodokoun et al. (2006). Average parasitism rates inflicted by *P. leucobasis* and *B. kriegeri* on *M. vitrata* larvae collected from the most commonly occurring wild host plants (Fabaceae) were 30.2 and 4.2%, respectively. In comparison on cowpea, parasitism rates were 5.6% for *P. leucobasis* and 4.9% for *B. kriegeri.*

Note that if two conditions exist, only region 1 is relevant to the evolution of resistance in *M. vitrata.* If no additional generations are permitted on wild hosts after cowpea cultivation ends in regions 2 and 3 and no reverse migration to region 1 occurs, then regions 2 and 3 actually play no role in long-term IRM, because individuals selected for resistance cannot contribute to the next year's population.

Standard Simulation Conditions. A flow diagram for the simulations is presented in Fig. 2. The model has a time-step of one generation with nine generations per year. The initial number of adults is 60,000 per ha moving to wild hosts in each region. Adults begin at Hardy–Weinberg equilibrium with an initial resistance-allele frequency of 0.001 for each resistance allele. Table 4 provides a list of variables and parameters. In the standard simulations, toxin survival is 0.01, resistance expression hh1 = hh2 = 0.1, and V = 0.5. The standard initial proportion of cowpea landscape that is Bt cowpea is 0.1 with increasing adoption for 5 yr. The model is simulated for 200 yr, and the year in which each allele frequency exceeds 50% is recorded.

The computer code written in C++ was verified by performing many tests on subroutines and the entire algorithm. Before simulating the results described below, we confirmed that the code calculated results logically.

We performed several sensitivity analyses to better understand how the dynamics of the modeled system behave over a reasonable range of parameter values. There are too many combinations of assumptions and conditions to analyze in one manuscript; therefore, we chose to focus on several conservative scenarios. The initial focus is on a single region with significant amounts of both wild hosts and cowpea and persistent populations of the pest (region 1). The second phase considers all three regions. Given the lack of return migration to region 1 and the small amount of natural refuge in the northern two regions, we do not expect the evolution of resistance to be faster in the combined area compared with region 1 by itself. Finally, we restrict the planting of Bt cowpea to regions 2 and 3, which makes region 1 a source of unselected pests.

We have modeled a set of scenarios that many would consider to be "worst case." No non-Bt cowpea is required to be planted or sold in the model. Thus, we only considered natural, nonstructured refuge scenarios. We assumed that seed will be saved by all farmers to be planted in the next year and that cross-pollination will occur. Thus, we not only simulate a seed mixture or blend, but even more risky mixtures with many patches of cowpea with intermediate tox-

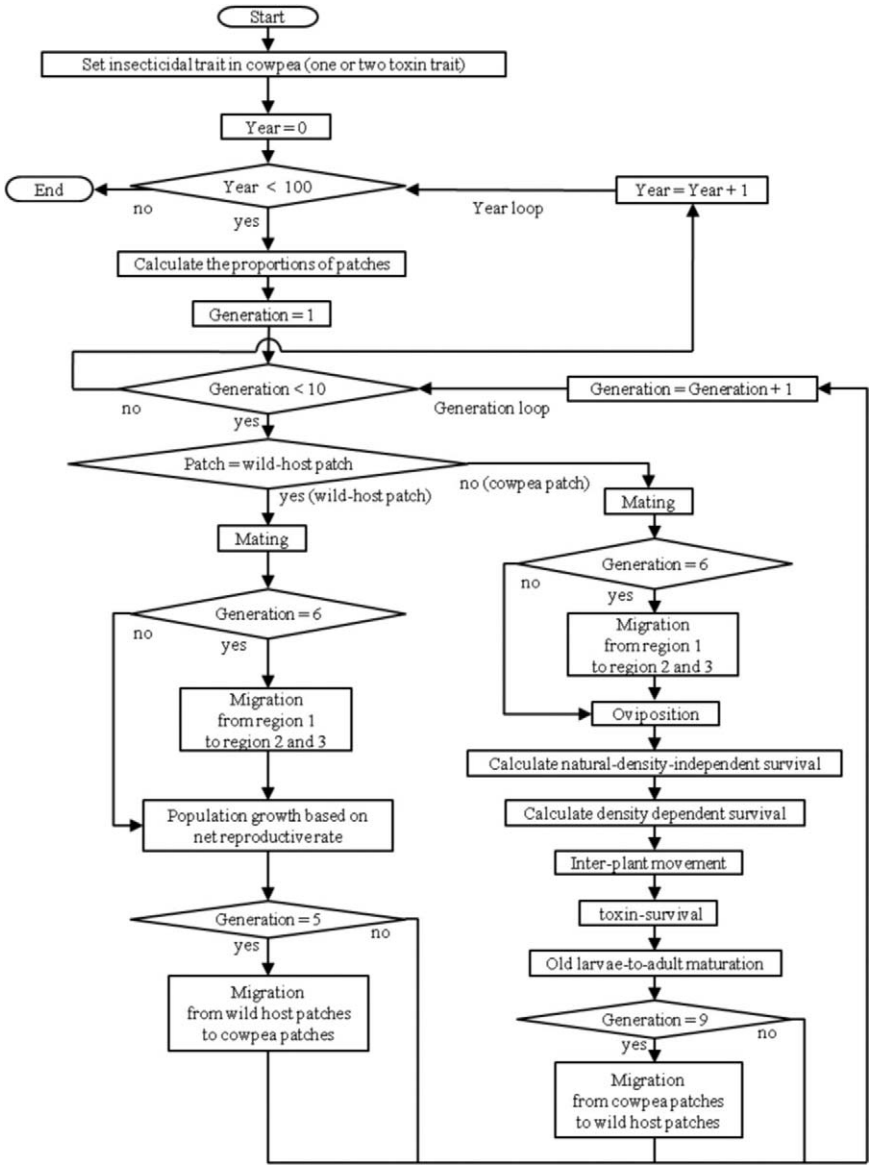

Fig. 2. Flow diagram for simulations of model.

icity. Therefore, additional differential selection between susceptible and heterozygous insects may occur in the simulations. Also, we assume that four generations of *M. vitrata* occur every year on cowpea; three generations may be realistic in some regions. Our standard assumption is that fitness costs in resistant insects do not exist. All of these factors have promoted faster evolution of resistance in the model.

Results

Bt Cowpea in Region 1. Fig. 3 and Table 5 present the sensitivity analyses for two of the most important factors influencing the evolution of resistance in insects (Onstad 2008): survival of homozygous susceptibles to toxin and dominance of resistant allele. These simulations are based on the simulation of dynamics and genetics only in region 1 without emigration of the pest. As expected, as dominance of resistance increases from recessive to dominant levels, evolution occurs more quickly. The results indicate that single-toxin Bt cowpea will not likely be adequate for controlling and delaying resistance in the southern-most region in west Africa (Fig. 3). For example, under standard conditions, (SN = 0.01, hh = 0.1) resistance evolves in 6 yr (Fig. 3). For pyramided Bt cowpea, as long as dominance hh1 and hh2 are both <0.5 and survival SN1 and SN2 are <0.01, a satisfactory pyramided Bt cowpea can be

Table 4. List of symbols used in model for variables, indices, and parameters

Type	Name	Standard value	Description
Variables	A		The no. of adults
	E		The no. of eggs
	F		The no. of female moths
	FM		The no. of mated female moths
	L		The no. of young larvae
	M		The no. of male moths
	ML		The no. of older larvae
	N		The no. of neonates
	TL		The total no. of young larvae per ha
	Z		The genotypic frequency of male mates
Indices	c		Region
	g		Maternal genotype
	i		Genotype
	m		Paternal genotype
	n		Type of natal cowpea patch
	r		Type of cowpea patch
	t		Generation
	w		Wild-hosts
Parameters	Cr	0	The proportion of cross-pollination
	DCW	0.5 (region 1) 0.05 (region 2) 0.01 (region 3)	The proportion of adults dispersing from cowpea to wild hosts
	DWC	0.5	The proportion of adults dispersing from wild hosts to cowpea
	h	.	The dominance of resistance allele
	hh	0.1	The dominance of resistance to the toxin in pure Bt cowpea plants
	MIG	0.36	The proportion of the Region 1 pop that migrates out of region
	NRR	2	The net reproductive rate on wild hosts
	P	.	The proportion of a type of cowpea patch
	Q	.	The proportion of land planted with new Bt cowpea.
	RT	.	The proportional reduction in toxin-survival rate
	Smax	.	The max toxin-survival rate
	Smin	.	The min. toxin-survival rate
	SN	0.01	The survival of a susceptible homozygote on a full dose of one toxin
	Stox	.	Toxin-survival rate
	SX	1	The survival of resistant homozygotes on cowpea
	Tr	0.1	The proportion of transgenic cowpea in year 1
	V	0.5	Probability of a larva leaving a plant

developed that delays resistance evolution at least 21 yr (Table 5).

With single-toxin Bt cowpea in region 1 (without emigration), results were sensitive to Bt-cowpea adoption rate, Tr. When we varied TR from 0.05 to 0.175 per year, the timing of resistance evolution decreased from 9 to 5 yr. With pyramided Bt cowpea, the pest is extirpated before evolution occurs. When we varied initial frequency of the resistance allele from 0.01 to 0.000001 the evolution of resistance ranged from 5 to 11 yr under all other standard conditions for a single-toxin Bt cowpea. However, this same range of initial allele frequencies with pyramided Bt cowpea always resulted in extirpation of the pest before evolution of resistance. In fact, in all other sensitivity analyses with pyramided Bt cowpea, the insect population always went extinct before resistance evolved.

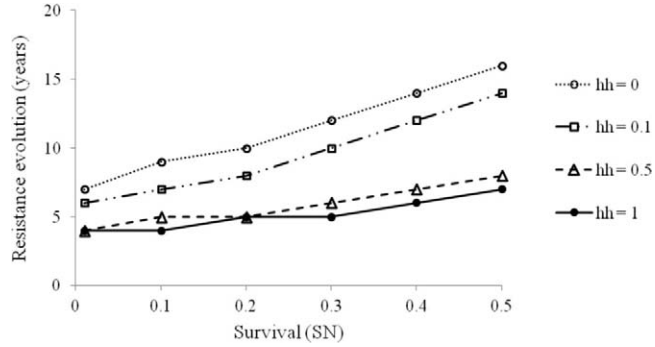

Fig. 3. Influence of toxin survival rate of homozygous susceptible larvae, SN (proportion surviving stage), and dominance of resistance allele (hh), on number of years required for resistance to evolve in *M. vitrata* to single-toxin-Bt cowpea in region 1 without emigration.

Table 5. Influence of toxin survival rate of homozygous susceptible larvae, SN, (proportion surviving stage) and dominance of resistance alleles, hh, on number of years required for resistance to evolve in *M. vitrata* to pyramided Bt cowpea in Region 1 without emigration

hh	SN						
	0.001	0.01	0.1	0.2	0.3	0.4	0.5
0	e	e	e	e	e	e	>200
0.01	e	e	e	e	e	113	123
0.1	e	e	e	e	51	23	26
0.25	e	e	e	51	15	12	14
0.5	23	23	15	9	8	8	9
0.75	13	12	8	6	6	7	8
1.0	7	7	6	6	6	6	7

Standard values of SN and hh are 0.01 and 0.1.

e indicates that the number of *M. vitrata* falls below 1.0 by 10^{-15} before resistance evolves.

Resistance evolves 3 yr later when population growth rate on wild hosts, NRR, for homozygous susceptible insects is increased to 2.1 from values 2.0, 2.001, or 2.01, which are all 7 yr with single-toxin Bt cowpea. This occurs because it affects the selection differential between resistant and susceptible insects. However, reducing NRR for resistant moths to 1.9 and keeping all other NRR = 2.0 had no effect on the results.

The effect of dispersal from wild hosts to cowpea, DWC, on resistance evolution is greater than that of dispersal from cowpea to wild hosts, DCW. Depending on DWC, resistance evolves 1–2 yr earlier or later with single-toxin Bt cowpea (Table 6).

Bt Cowpea in Three Regions. Under standard conditions with single-toxin Bt cowpea grown in all three regions, resistance evolves in 6 yr in region 1 and in 7 yr in the other two regions. The value for region 1 is no different from that shown in Fig. 1. For single-toxin Bt cowpea, model results were not sensitive to 1) regional migration rate, MIG, 2) proportion of wild hosts in regions 2 and 3, and 3) the exponent for Stox used in equations 6–8. Results were also the same with 0 and 1% cross-pollination. Thus, our complex model can be simplified. With pyramided Bt cowpea planted in all regions, extinction occurs before resistance evolves.

Bt Cowpea in Regions 2 and 3. When cowpea is grown in all regions but single-toxin-Bt cowpea is used only in regions 2 and 3, and moths emigrate from region 1, evolution of resistance is delayed by >15 yr in only a few scenarios with SN1 < 0.1 (Table 7). For region 2, the resistance allele must be completely recessive. For region 3 dominance must be incompletely recessive, hh1 ≤ 0.25. Genotypic frequencies of adults migrating from the region 1 to the other regions are constant over time because there is no selection pressure and fitness cost for Bt resistance in region 1. More resistant moths can persist in region 2 compared with region 3, because 5% (region 2) versus 1% (region 1) of insects surviving in Bt cowpea move to wild hosts at the end of cropping season. For this reason, migrants from Region 1 more effectively delay resistance in the region 2 than region 3.

If cowpea expressing two Bt toxins is planted only in regions 2 and 3, resistance evolves after 200 yr for all cases with hh ≤ 0.75. The only exception is for region 2 with hh = 0.75 and SN = 0.3, in which case the allele frequencies exceed 0.5 in 14 yr. Clearly, pyramided Bt cowpea is less risky than single-toxin Bt cowpea when planted in the two northern regions.

Table 8 shows the sensitivity of results with single-toxin Bt cowpea to changes in migration rate, MIG. For region 2, as MIG increases from 0.1 to 0.5, resistance evolves in 9–16 yr. For region 3, the standard time is >200 yr with MIG = 0.36, but with MIG = 0.1, the durability declines to 27 yr (Table 8). Given the importance of the region 1 in supplying susceptible moths to the northern regions, this sensitivity is not surprising. Because of the extremely long delays in evolution with pyramided Bt cowpea, sensitivity in those cases could not easily be determined.

Table 6. Influence of moth dispersal rates from wild to cowpea, DWC, and from cowpea to wild hosts, DCW, on number of years required for resistance to evolve in *M. vitrata* to single-toxin Bt cowpea in Region 1 without emigration

DWC	DCW			
	0.25	0.5	0.75	1.0
0.25	8	8	8	8
0.5	7	7	6	6
0.75	6	6	6	6
1.0	6	6	5	5

Table 7. Influence of toxin survival rate of homozygous susceptible larvae, SN, and dominance of resistance allele, hh, on number of years required for resistance to evolve in *M. vitrata* to single-toxin Bt cowpea used only in the northern Regions 2 and 3

hh	Region	SN						
		0	0.0001	0.001	0.01	0.1	0.2	0.3
0	2	47	47	47	52	>200	>200	>200
0.1		13	13	13	13[a]	19	47	>200
0.25		8	8	8	8	10	17	>200
0.5		6	6	6	6	7	10	>200
0.75		5	5	5	5	6	8	17
1.0		5	5	5	5	6	7	12
0	3	>200	>200	>200	>200	>200	>200	>200
0.1		>200	>200	>200	>200[a]	>200	>200	>200
0.25		17	17	17	18	>200	>200	>200
0.5		8	8	8	8	13	>200	>200
0.75		6	6	6	6	9	>200	>200
1.0		6	6	6	6	8	>200	>200

[a] Indicates results with standard version of model.

Table 8. The effect of migration from Region 1, MIG, on number of years required for resistance to evolve in *M. vitrata* to single-toxin Bt cowpea used only in the northern Regions 2 and 3

Region	MIG					
	0.1	0.2	0.3	0.36	0.4	0.5
2	9	11	12	13[a]	14	16
3	27	187	>200	>200[a]	>200	>200

[a] Indicates results with standard version of model.

Discussion

We have modeled a set of scenarios that many would consider to be conservative because the conditions promote faster evolution of resistance in the model. We have shown that natural refuge and traditional cowpea crops can provide enough refuge to delay resistance. The results indicate that as long as a pyramided Bt cowpea can be developed, seed saving and reliance on natural refuge are not major problems for resistance management. Furthermore, it is possible that one or both toxins in the pyramid may not need to be high dose for evolution to be delayed >15–20 y (Table 5) if the Bt cowpea is deployed in regions where *M. vitrata* is endemic. It is possible that one superior trait could be combined with a less effective trait (providing survival >0.01), but more research is needed to measure this approach for planting pyramided Bt cowpea. Note that we assume that *M. vitrata* spends four generations per year on cowpea; therefore, a 20-yr delay in evolution is equivalent to 80 generations.

How low can the mortality caused by Bt cowpea be to delay resistance evolution 15–20 yr? Once the region where Bt cowpea will be planted and the dominance of resistance is either assumed or becomes known, Tables 5 and 7 can be used to select a target for the dose of toxin and the associated mortality engineered in the Bt cowpea.

Gould et al. (2006) and Roush (1998) used abstract models to study insect resistance management for toxin pyramids in plants. Roush demonstrated that for pyramids to be effective they should use toxins causing very high mortality. Gould et al. (2006) concluded that fitness costs (i.e., the reduced fitness of resistant phenotypes on nontransgenic crop relative to susceptible phenotypes), can be important for resistance evolution and that planting single and multiple-toxin crops near each other can contribute to faster evolution of resistance to both toxins. Roush (1998) drew a similar conclusion about the negative influence of single-toxin crops in his analysis of impurities in insecticidal seeds. In our study, we assume that either single-toxin or pyramided Bt cowpea (but not both) will be purposefully deployed in west Africa. Furthermore, with seed saving and cross-pollination, some low-toxicity Bt cowpea will exist in the landscape with high toxicity Bt cowpea. However, these factors were not important in our simulations.

If >9 generations of *M. vitrata* occur each year on wild hosts and if resistant individuals suffer fitness costs while infesting wild hosts, then resistance to Bt cowpea should be delayed more than what we have estimated using the standard model. However, it is important to note that unlimited amounts of Bt cowpea can be planted in areas where *M. vitrata* does not persist on wild hosts and from which no return migration occurs to the south.

Although cowpea is grown in lower quantities in the endemic zone for *M. vitrata* as compared with the more northerly nonendemic regions, based on our results there will be a need to develop a logical action plan in regards to where the Bt cowpea crops will be actively promoted. For example, if a single-toxin Bt cowpea is ultimately released, deployment plans, including seed distribution systems, should focus on those regions where *M. vitrata* are not endemic. However, if a two-toxin Bt cowpea is developed and released, active distribution of seed material into areas where *M. vitrata* is also endemic may not be problematic from an IRM prospective.

This modeling effort represents a first step in addressing potential issues associated with IRM associated with Bt cowpeas in west Africa. Future research should focus on measuring migration from south to north. In addition, more work is needed to measure the contribution of *M. vitrata* on wild hosts. Baoua et al. (2011) found abundant larvae on one species of the wild hosts, but its value as a source of adults is uncertain. Our results indicate that a complex model is not needed for predicting evolution of resistance to Bt cowpea. Cross-pollination is insignificant and so most of equations in Tables 1 and 2 are not needed. It is also important to note that many of our assumptions represent a worst-case scenario. Thus, if the adoption rate of Bt cowpea is slower than we assumed (which is a distinct possibility) or wild refuges contribute to a greater amount of the *M. vitrata* population, then resistance would be further delayed. Also, we recommend that deployment of a single Bt cowpea should be focused in areas where *M. vitrata* is not endemic. If a two-toxin Bt cowpea can be developed, it has the potential to be used across a much greater range and in many more agroecological conditions. However, our results suggest that even when a worst-case scenario is assumed, there are deployment strategies that can be taken for Bt cowpea that can both help cowpea farmers in west African and ensure an effective IRM strategy for this crop-pest system.

Acknowledgments

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