

# Modeling the Integration of Parasitoid, Insecticide, and Transgenic Insecticidal Crop for the Long-Term Control of an Insect Pest

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**ABSTRACT** The tools of insect pest management include host plant resistance, biological control, and insecticides and how they are integrated will influence the durability of each. We created a detailed model of the population dynamics and population genetics of the diamondback moth, *Plutella xylostella* L., and its parasitoid, *Diadegma insulare* (Cresson), to study long-term pest management in broccoli *Brassica oleracea* L. Given this pest's history of evolving resistance to various toxins, we also evaluated the evolution of resistance to transgenic insecticidal Bt broccoli (expressing Cry1Ac) and two types of insecticides. Simulations demonstrated that parasitism provided the most reliable, long-term control of *P. xylostella* populations. Use of Bt broccoli with a 10% insecticide-free refuge did not reduce the long-term contribution of parasitism to pest control. Small refuges within Bt broccoli fields can delay evolution of resistance >30 generations if resistance alleles are rare in the pest population. However, the effectiveness of these refuges can be compromised by insecticide use. Rainfall mortality during the pest's egg and neonate stages significantly influences pest control but especially resistance management. Our model results support the idea that Bt crops and biological control can be integrated in integrated pest management and actually synergistically support each other. However, the planting and maintenance of toxin-free refuges are critical to this integration.

**KEY WORDS** simulation, broccoli, diamondback moth, *Plutella xylostella*, *Diadegma insulare*

Three major tactics for controlling agricultural insect pests are biological control, insecticide use, and host-plant resistance using transgenic insecticidal crops. Integrating these in some combination to improve long-term integrated pest management (IPM) has been a goal of many entomologists. Long-term IPM depends on adequate insect resistance management (IRM) to either delay resistance evolution or at least mitigate the consequences of resistance evolution (Onstad 2008). Because pests can evolve resistance to insecticides and transgenic insecticidal crops, any good long-term strategy will be based on an approach that balances short-term lethal effects with variation in type and level of control. Gassmann et al. (2009), Lundgren et al. (2009), and Onstad et al. (2011) summarize many of the issues involving integration of

tactics within IRM and the compatibility of natural enemies with transgenic insecticidal crops.

Transgenic insecticidal crops should be considered as a particular form of host plant resistance (Onstad and Knolhoff 2008). As such, they are subject to the same conceptual models for tritrophic interactions of natural enemies on resistance evolution in the pest species. This question was first taken up by Gould et al. (1991) in their conceptual and mathematical models on tritrophic interactions of a plant, an herbivore, and a natural enemy. They realized that hypotheses derived from the deterministic models would be significantly influenced by a variety of interacting ecological, behavioral, and genetic processes acting over a single or multiple generations. Their simplest conclusion was that natural enemies that increase differential fitness between susceptible and resistant phenotypes on host plants will accelerate evolution of resistance; those that decrease the differential will delay resistance.

Heimpel et al. (2005) extended the conceptual work of Gould et al. (1991) and modeled the influence of egg mortality on the high-dose/refuge strategy for IRM. They modeled various levels and forms of pest egg mortality: density independence, positive density dependence, and inverse density dependence. Their results indicated that both the magnitude and form of egg mortality can influence the rate of resistance evo-

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lution, but the importance of egg mortality depends on other ecological processes in the pest population.

Arpaia et al. (1997) investigated predation on the Colorado potato beetle, *Leptinotarsa decemlineata*, on transgenic insecticidal potato plants in greenhouse and field studies. They included predation rates in a mathematical model to simulate impact of natural enemies on the evolution of resistance by *L. decemlineata* to transgenic insecticidal potato. Simulations also included refuges of conventional potato plants. Their results showed that predation could decrease the rate of resistance evolution. Mallampalli et al. (2005) performed field studies to calibrate a simulation model of *L. decemlineata* on transgenic insecticidal potatoes to determine the influence of predation on IRM. They discovered that different prey species of a generalist predator that eats *L. decemlineata* have different effects on evolution of resistance in *L. decemlineata* to transgenic insecticidal potato: one prey species delays resistance while the other accelerates the evolution of resistance.

Diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is a major cosmopolitan pest of *Brassica* (Talekar and Shelton, 1993, Grzywacz et al. 2010, Zalucki et al. 2012). It has evolved resistance to many insecticides (Grzywacz et al. 2009), including foliar applications of *Bacillus thuringiensis* (Berliner) Bt (Tabashnik et al. 1990, Shelton et al. 1993). Several transgenic insecticidal *Brassica* crops have been developed to add one more tactic for diamondback moth IPM (Shelton et al. 2008) and they have been used to study factors involved in the evolution of resistance to Bt plants.

Chilcutt and Tabashnik (1999) simulated a model of the interactions of a foliar insecticide containing Bt and a parasitoid in the control of diamondback moth. They also modeled the population genetics of diamondback moth and its evolution of resistance to Bt. They concluded the use of parasitoids could slow the evolution of resistance to Bt by decreasing the number of generations in which insecticide treatment is required, but this would not directly apply to Bt plants with continuous expression.

The goals of this project are to increase our understanding of the effect of Bt plants and conventional insecticides on the persistence and population dynamics of natural enemy and diamondback moth populations and how these may affect the rate of evolution of resistance to Bt plants and insecticides in diamondback moth. In addition, we explore how these effects may be influenced by an environmental factor (rainfall) that has been shown to be a major mortality factor for diamondback moth (Talekar and Shelton 1993). In this article, we focus on a parasitoid of the diamondback moth. In a companion article, we will investigate the interaction of predation with other components of the system.

### Materials and Methods

This article describes a model of the population dynamics and genetics of diamondback moth and the

population dynamics of its parasitoid, *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), an important natural enemy that regulates diamondback moth populations (Shelton 2004). It uses a generational time step in a manner similar to the model of Chilcutt and Tabashnik (1999). The model calculates changes in several life stages and genotypes over multiple generations. The functions, parameters, and analytical approach used are as follows.

This model has functions that are calculated once per generation. The parasitoid and host have synchronized life cycles with similar durations. Harcourt (1986) concluded that parasitism by *D. insulare* was weakly density-dependent and no other factor contributing to population fluctuations was density-dependent.

We simulate a landscape divided into two blocks of broccoli: one with Bt broccoli, the other a refuge of conventional broccoli. The Bt broccoli expressed Cry1Ac from the bacterium, *Bacillus thuringiensis* (Shelton et al. 2008). In the model, block or patch one is refuge and block or patch two is the Bt broccoli. In some scenarios, we simulate insecticide applications in the refuge. Adults of all species can move among blocks, but larvae cannot. Such movement is realistic since Schellhorn et al. (2008) observed both diamondback moth and *Diadegma* adults dispersing >100 m. The proportion of Bt broccoli plants in the landscape is T, and the proportion of refuge is (1-T). The total number of plants in the landscape is TP with NP(1) and NP(2) representing number for each patch or block.

**Population Genetics of Diamondback Moth.** We assume that mating is random among phenotypes and across blocks of broccoli (Chilcutt and Tabashnik 1999). Females mate once and males can mate multiple times (Wang et al. 2005). The diamondback moth population has one locus for resistance to Bt broccoli and one for resistance to a synthetic insecticide. Wild type alleles are S and A for the former and latter loci. Alleles conferring resistance in each case are R and B. We assume that the autosomal, di-allelic loci are independent. Liu et al. (2011) found no effects of diamondback moth genotypes on parasitism. We assume that no mutations occur after the start of simulations.

**Dispersal of Female Diamondback Moth.** Mating occurs after dispersal (Chilcutt and Tabashnik 1999). Because our primary focus is on small areas, we assume that females can move anywhere in the modeled landscape before mating and during oviposition. For simplicity, we assume that the proportion of females ovipositing in a given block of broccoli is equal to the proportional area of that block in the landscape.

**Diamondback Moth Oviposition.** Harcourt (1986) reported that fecundity declines with age of cabbage plant and protein content of cabbage leaves. Based on the work of Harcourt (1986) and the review by Talekar and Shelton (1993), we chose 150 eggs per female as the standard fecundity in the model. A Mendelian function determines proportion of offspring in each genotype given genotypes of parents.

Liu et al. (2012b) found no direct effects of Bt broccoli or spinosad on fecundity nor any ovipositional preference by females for spinosad-treated or Bt broccoli. However, susceptible diamondback moth females oviposited fewer eggs on plants sprayed with lambda-cyhalothrin than on unsprayed plants. At the field dose of 80 ppm, oviposition was observed to be 13% of normal on lambda-cyhalothrin sprayed plants (Liu et al. 2012b). To mimic this effect in the model, we kill 87% of the females attempting to oviposit in the sprayed block.

**Survival of Immature Stages of Diamondback Moth.** Harcourt (1986) concluded that the primary mortality factors for immature stages in his environments (other than insecticides) were rainfall harming neonates and parasitism affecting larvae. Annamalai et al. (1988) demonstrated that rainfall can kill both neonates and eggs. Neonates that have just hatched or are in the process of hatching after a rain event are drowned by the film of water before they are able to enter a leaf. Because the eggs and neonates may be either knocked off plants or are drowned by the rainwater and because the mortality occurs before any other mortality during the immature stage, we applied the mortality to the egg stage.

Although predation and parasitism occur during the larval stage, we omitted predation from this model for three reasons. First, the vast majority of the literature has documented the importance of parasitoids as the primary natural enemy type regulating populations of *P. xylostella* (Shelton 2004, Sarfraz et al. 2005). Second, the modeled system would have been too complex if predation had been included along with insecticide mortality, insecticidal crop mortality, rainfall mortality, and parasitism. Third, our original interest was in greenhouse systems in which the type of natural enemy can be selected. We are preparing a second model incorporating predation and without parasitism for future publication.

**First Instar Diamondback Moth.** We assume that toxin mortality because of either Bt broccoli or a synthetic insecticide occurs before parasitism. The following equation calculates the density of young diamondback moth larvae per plant LY in patch h and genotype g in generation t using a function of total adult density N in the previous generation.

$$\begin{aligned}
 LY(t,h,g) = & SL(h,g) \times Srf \times (1/Np(h)) \\
 & \times 0.5 \times F \times \sum_{g'=1}^9 \sum_{g''=1}^9 N(t-1, h, g') \times M(g, g', g'') \\
 & \times P(t-1, g'') \quad [1]
 \end{aligned}$$

where SL(h,g) is the probability of survival for LY because of toxin, NP is number of plants in patch h, and F is the fecundity. The parameter Srf is egg survival because of rainfall, which was estimated to be 0.52 in field studies (Harcourt 1986) and one for populations not subjected to rainfall. Constant 0.5 is sex ratio. M(g, g', g'') is the Mendelian function and P is the proportion of males in genotype g'.

**Table 1. Survival of *P. xylostella* larvae on Bt broccoli or insecticide-treated refuge broccoli in the model according to genotype**

Genotype	Bt broccoli	Refuge treated with	
		LTI <sup>a</sup>	HTI <sup>a</sup>
SSAA	0.001	0.11	0.03
SSAB	0.001	0.22	0.14
SSBB	0.001	0.80	0.80
SRAA	0.001	0.11	0.03
SRAB	0.001	0.22	0.14
SRBB	0.001	0.80	0.80
RRAA	1.0	0.11	0.03
RRAB	1.0	0.22	0.14
RRBB	1.0	0.80	0.80

<sup>a</sup> LTI and HTI are less toxic and highly toxic insecticides, respectively.

**Insecticide Mortality.** Foliar insecticides are the main control method for diamondback moth on broccoli and, based on our experience, are often applied every 10-d by broccoli growers. Two commonly used insecticides are lambda-cyhalothrin and spinosad, each with a different mode of action and level of toxicity to diamondback moth (see below). In our model, we assume that young larvae are targeted by scheduled or density-threshold based applications. Liu et al. (2012b) measured the survival of young larvae during an 11-d residue period after applications of spinosad and lambda-cyhalothrin under laboratory conditions. By day 11, significant numbers of diamondback moth larvae were surviving contact with the residues. All genotypes have 100% survival on untreated refuge. Over the 10-d residue period, we expect the insecticides can kill both stages of larvae (young and old); however, given our assumption of an application every 10 d, no older larva could avoid exposure as a young larva. Therefore, we avoid erroneous redundant killing by permitting insecticide mortality only on young larvae (and adults as noted above). The mean survival rates for larvae on Bt broccoli and in treated refuges are based on the data of Liu et al. (2012b) for lambda-cyhalothrin and spinosad (Table 1). To emphasize the toxicity, but not the active ingredient, of insecticide we define the two modeled insecticides as highly toxic (HTI) and less toxic (LTI).

**Density-Dependent Survival.** We limit the diamondback moth population using a simple density-dependent survival function,  $0.4 \exp(-0.05TLY(t,h))$ . The two parameters were chosen to allow moderate cycling of the population densities over generations in simulations without chemical or biological control. Without rainfall causing egg and neonate mortality, our function allows the population to cycle between 0.03 and 58 adults per 20 non-Bt broccoli plants with a maximum of 219 young larvae per plant. We have observed maxima close to 200 per plant in greenhouse cages (unpublished data). With rainfall mortality, a maximum of 114 young larvae per plant is simulated and the adult density fluctuates between 3 and 59 per 20 plants.

**Parasitism.** Sarfraz et al. (2005) reviewed the biology of *D. insulare*. Xu et al. (2001) concluded that *D. insulare* is a good searcher for diamondback moth larvae, and Sarfraz et al. (2010) found that the female parasitoids are distributed spatially according to the spatial distribution of diamondback moth density. Harcourt (1986) studied diamondback moth and *D. insulare* in southern Ontario using 74 generational life tables. Harcourt (1986) and Xu et al. (2001) observed parasitism increasing over the 4–5 diamondback moth generations each year in New York or Ontario, Canada, respectively. Harcourt (1986) states that the parasitoid attacks the first three larval instars, but Putnam (1968) in a study on rape plants observed few if any first instars attacked, and 23% of fourth instars attacked over 48 h. Second and third instars were equally attacked over 2 d: 45% of 100 larvae. Xu et al. (2001) observed 22% of third instars and 31% of fourth instars parasitized.

We used several sources to derive a function that calculates proportion parasitized each generation. Bolter and Laing (1983) concluded that multiple parasitism and superparasitism are insignificant in *D. insulare* populations. Putnam (1968) observed the proportion parasitized over 2 d was inversely proportional to density of diamondback moth larvae, where the first number is density and second is parasitism: (50, 0.78), (100, 0.70), (200, 0.57), and (400, 0.38). Putnam (1968) concluded that the maximum fecundity was 516 offspring/female parasitoid when the parasitoids were exposed to 1,300 diamondback moth larvae over 1 mo. Bolter and Laing (1983) concluded that fecundity of *D. insulare* could be higher than 800 eggs/female when exposed to circa 2,000 diamondback moth larvae until death of the parasitoid. Over 48 h, Putnam (1968) observed each *D. insulare* parasitizing a total of 47% of diamondback moth larvae when 200 diamondback moth larvae were spread over 15 rape plants in a greenhouse cage with a 0.97 m<sup>2</sup> floor at 21–24°C. Xu and Shelton (2001) observed 95% of diamondback moth larvae parasitized in greenhouse cages of 0.17 m<sup>3</sup>.

We chose the simplest function that mimics several of these observations:  $0.05 + 0.95\exp(-700 Da(t - 1)/(TLY(t,h) + 1))$  is the proportion of larvae escaping parasitism. The variable TLY is the sum over all genotypes of the density of young larvae per plant, and Da is the density of female parasitoids per plant in the landscape. As female parasitoid density increases, the proportion of parasitism declines exponentially and, as larval density increases, the proportion increases. For example, with Da = 1 and TLY = 150 per plant on one plant, the proportion parasitized is 0.95. With Da = 0.1 and TLY = 150 per plant on 10 plants, 35% of all young larvae on all plants in that patch are parasitized by the single parasitoid, which effectively produces 525 offspring during its lifespan, very close to the 516 found by Putnam (1968).

Chen et al. (2008) observed equal parasitism on all diamondback moth genotypes tested on conventional, untreated broccoli plants. Chen et al. (2008) also found that parasitism is unaffected by diamondback moth feeding on Bt-broccoli when resistant Bt-dia-

mondback moth are used. Liu et al. (2012a) observed one-third as much parasitism on lambda-cyhalothrin treated broccoli as on nontreated plants, but no effect was observed with spinosad. Hill and Foster (2003) observed greater parasitism in plots treated with spinosad than in untreated plots. Therefore, we reduced parasitism for a HTI.

The density of older nonparasitized diamondback moth larvae (second to fourth instars) per plant LO in patch h, genotype g and generation t is

$$LO(t,h,g) = LY(t,h,g) \times 0.4\exp[-0.05TLY(t,h)] \\ \times (1 - p_{max} \times \{1 - \exp[-700Da(t - 1)/ \\ (TLY(t,h) + 1)]\}) \quad [2]$$

where p<sub>max</sub> is the maximum proportion that can be parasitized each generation and Da is the number of female parasitoids per plant after movement in the landscape. The value of p<sub>max</sub> is 0.95, except on plants treated with a HTI when it is 0.31. The variable LY is multiplied by two survival factors specific to each patch h: 1) the proportion surviving competition for food based on density of young larvae per plant, and 2) the probability not being parasitized.

**Parasitoid Adults.** The densities of adult parasitoids are calculated with the following three equations. The density of parasitoids before movement away from the block in which they emerge from dead larvae is

$$Db(t,h) = SP(h) \times 0.4\exp[-0.05TLY(t,h)] \\ \times TLY(t,h) \times p_{max} \times \{1 - \exp[-700Da(t - 1)/ \\ (TLY(t,h) + 1)]\} \quad [3]$$

where SP(h) is the probability of survival for adult parasitoids when exposed to synthetic insecticides. We assume that an immature parasitoid is killed before or after emergence from an intoxicated diamondback moth larva or by contact with contaminated surface of broccoli. Chen et al. (2008) evaluated the effects of several toxins on *D. insulare*, including Cry1C Bt broccoli plants, the purified Cry1C toxin applied as a spray, and the commonly used insecticides spinosad and lambda-cyhalothrin soon after treatments were applied. Strains of *P. xylostella* that were resistant to Cry1C and to the commonly used insecticides were used to minimize host-quality mediated impacts and to assess direct impact (toxicity) on the parasitoid. Their results indicated that Cry1C Bt broccoli plants and the purified Cry1C toxin had no direct toxicity to *D. insulare*. Chen et al. (2008) and Hill and Foster (2000) observed no survival of adult parasitoids directly treated with spinosad. Chen et al. (2008) observed no survival of adult parasitoids directly treated with lambda-cyhalothrin. However, Xu et al. (2004) observed increased survival on leaves as time passed in a 10-d residue study. On the 10th day, survival of *D. insulare* adults was 93 and 43% for spinosad and lambda-cyhalothrin treated leaves. Therefore, mean survival over a 10-d residue period is assumed to be 0.65 or 0.22 for *D. insulare* adults encountering LTI and HTI, respec-

tively, in the refuge. Otherwise,  $SP(1) = 1$ , and  $SP(2)$  is always 1.

$$Da(t) = 0.5 \times [NP(1) \times Db(t,1) + NP(2) \times Db(t,2)] / TNP \quad [4]$$

where TNP is total number of plants in landscape and NP is the number in each patch. The sex ratio is 0.5 (Sarfraz et al. 2005).

**Diamondback Moth Adults.** Finally, we calculate the total number of diamondback moth adults N in patch h (not per plant) developing from larvae LO on each plant.

$$N(t,1,g) = SI(1,g) \times (1 - T) \times [NP(1) \times LO(t,1,g) + NP(2) \times LO(t,2,g)]$$

$$N(t,2,g) = T \times [NP(1) \times LO(t,1,g) + NP(2) \times LO(t,2,g)] \quad [5]$$

when a highly toxic insecticide, such as lambda-cyhalothrin, is applied to the refuge in the model, SI equals 0.13 for homozygous susceptible (-AA) and heterozygous moths (-AB) (Chen et al. 2008). For susceptible adults exposed over a 10-d residue period to a less toxic insecticide, we make  $SI = 0.48$  to match the relative survival observed with diamondback moth larvae. For other phenotypes or without insecticide use,  $SI = 1$ .

**Model Analysis.** The model was programmed in C# on a Dell computer. The landscape consists of 20 broccoli plants. The standard simulations had the following initial conditions: 20 total diamondback moth adults with genotypes distributed according to the Hardy-Weinberg formula and three mated female adult parasitoids. Unless otherwise indicated, the initial resistance allele frequencies are both 0.001. The standard initial density of female parasitoids is three per 20 plants.

We calculated the model for a maximum of 100 generations and recorded the allele frequencies and the densities of all diamondback moth genotypes and determined when the diamondback moth became resistant to the toxins (50% allele frequency). We recorded the proportion parasitized in each patch for the generation during which the R allele frequency exceeded 0.05 and at the end of the simulation. Extirpation occurs if the density of either species drops below  $10^{-12}$ .

### Results

**Model Without Bt Broccoli.** When no Bt broccoli is planted and no insecticide is used, the modeled diamondback moth population does not evolve. The density of adults in the 20-plant landscape fluctuates from 0.03 to 58 adults without rainfall mortality of eggs and neonates and 3-59 adults with rainfall mortality. The density-independent mortality reduces the density of young larvae before competition-based mortality takes effect.

**Table 2. Parasitism by *Diadegma insulare* and diamondback moth densities under various scenarios without Bt broccoli over first 10 generations**

Insecticide	Parasitoids	Rainfall <sup>a</sup>	Diamondback moth adults <sup>b</sup>	Parasitism rate <sup>c</sup>
None	No	No	20.9 (21.1)	
		Yes	28.1 (21.1)	
	Yes	No	2.5 (0.6)	0.93 (0.07)
		Yes	1.3 (1.5)	0.94 (0.02)
Less toxic	No	No	23.8 (5.4)	
		Yes	12.8 (14.8)	
	Yes	No	0.34 (0.67)	0.04 (0.45)
		Yes	0.07 (0.20)	0.03 (0.42)
Highly toxic	No	No	11.8 (18.5)	
		Yes	12.1 (18.7)	
	Yes	No	12.8 (22.4)	0.09 (0.13)
		Yes	8.8 (15.1)	0.07 (0.12)

In all four scenarios for each insecticide, diamondback moth evolves resistance in nine and four generations, respectively, to less toxic and highly toxic insecticide, as defined in the text.

<sup>a</sup> Rainfall mortality of diamondback moth eggs at 48%, from Harcourt (1986).

<sup>b</sup> Mean and SD of diamondback moth adults per 20 broccoli plants.

<sup>c</sup> Mean and SD of proportion of young larvae parasitized each generation.

Table 2 presents two aspects of diamondback moth population dynamics that permit us to evaluate parasitism and diamondback moth densities under various scenarios without Bt broccoli. As stated above, the diamondback moth population cycles over the first 10 generations, without any chemical or biological control, at densities that are likely damaging to the crop (i.e., 21-28 adults per 20 plants on the average). Parasitism of 93-94% of young larvae significantly reduces the mean adult density per generation. In fact, the adult density is relatively stable near 2.5 adults per 20 plants without insecticide mortality (Table 2).

When insecticides are applied to all the non-Bt broccoli plants and the initial B allele frequency is 0.001, resistance evolves quickly to either type of insecticide and is not influenced by parasitism or rainfall mortality of diamondback moth eggs and neonates. Because of the lack of a refuge, resistance evolves in four and nine generations with the constant use of HTI and LTI insecticides, respectively.

The integrated management of diamondback moth using parasitism and LTI maintains the mean diamondback moth adult density at the lowest levels with or without rainfall (Table 2). With an LTI, parasitism begins the 10 generation period at 95% but declines significantly after the first 3 yr producing an average of 3-4% (Table 2) because of long-term insecticidal mortality of diamondback moth and harm to the parasitoid. Thus, with an LTI, parasitism contributes to the pest control in the short term (IPM) but does not delay the evolution of insecticide resistance, which occurs in generation nine with or without parasitism. Because diamondback moth evolves resistance to the HTI within four generations, the mean pest density is only about half the densities occurring without any control. Because diamondback moth evolves so quickly to the HTI, the lower parasitism in this case is mostly because of direct toxicity to parasitoid.

**Table 3.** Number of generations required for the frequency of the allele conferring resistance to Bt broccoli to exceed 50% when no insecticide is applied to the refuge, in presence or absence of rainfall mortality of eggs and larval parasitism by *Diadegma insulare*, for different initial resistance frequencies for Bt (0.001 and 0.01)

Refuge size <sup>a</sup>	Rainfall mortality				No rainfall mortality			
	No parasitoids		Parasitoids		No parasitoids		Parasitoids	
	0.001	0.01	0.001	0.01	0.001	0.01	0.001	0.01
0.05	55	7	59	9	32	5	54	7
0.10	75	10	>100	15	37	6	>100	13
0.25	92	13	>100	39	46	7	>100	36

<sup>a</sup> Proportion of broccoli landscape.

**Bt Broccoli Without Insecticides.** When Bt broccoli is planted but no insecticide is used in refuge, as refuge size increases or initial R allele frequency decreases, the time to resistance increases (Table 3). Evolution of resistance to Bt broccoli is likely to occur more slowly in fields with significant rainfall than when there is none (Table 3). Without parasitoids in the environment, Bt broccoli as 90% of the crop reduces mean diamondback moth densities from over 21 adults per 20 plants (Table 2) to a relatively constant density of circa five adults (Table 4).

Parasitism always delays the evolution of resistance to Bt broccoli, especially when the refuge size is large (Table 3). The delays are much greater if rainfall mortality of diamondback moth eggs and neonates is not significant. That is, rainfall only slightly slows resistance if there is parasitism, but can delay resistance almost as much as parasitism in its absence, and vice versa (e.g., compare “no parasitoids” at an initial gene frequency of 0.001, with and without rainfall; the delays are on the order of 70–100% depending on refuge size, and only a bit less than parasitoids in the absence of rainfall). The benefits of rainfall and parasitism in

**Table 4.** Diamondback moth densities over first 10 generations under various scenarios with Bt broccoli and a 10% refuge, with and without larval parasitism by *Diadegma insulare* and rainfall mortality of diamondback moth eggs

Insecticide	Parasitoids	Rainfall <sup>a</sup>	Diamondback moth adults <sup>b</sup>	Parasitism rate <sup>c</sup>
None	No	No	5.4 (1.3)	
		Yes	4.7 (0.0)	
Less toxic	Yes	No	0.23 (0.34)	0.40 (0.41)
		Yes	0.07 (0.16)	0.35 (0.42)
	No	No	0.57 (1.4)	
		Yes	0.32 (0.86)	
Highly toxic	Yes	No	0.02 (0.07)	0.23 ext [8]
		Yes	0.01 (0.04)	0.20 ext [7]
	No	No	1.6 (4.1)	
		Yes	0.1 (0.3)	
Yes	No	0.83 (2.0)	0.07 ext [8]	
	Yes	0.08 (0.22)	0.06 ext [7]	

<sup>a</sup> Rainfall mortality of diamondback moth eggs at 48%, from Harcourt (1986).

<sup>b</sup> Mean and SD of diamondback moth adults per 20 broccoli plants.

<sup>c</sup> Mean and SD of proportion of young larvae parasitized each generation in refuge; ext(X) symbolizes extirpation of parasitoid pop in X generations.

**Table 5.** Number of generations required for the resistance allele frequencies to exceed 50% when an insecticide is applied to the 10% refuge for Bt broccoli and rainfall mortality occurs in the model

Initial frequencies of R and B	No parasitoids		Parasitoids	
	LTI <sup>a</sup>	HTI <sup>a</sup>	LTI	HTI
0.001, 0.001	55, 10	6, 39	55, 10	6, 42
0.001, 0.01	68, 6	42, 4	73, 6	42, 4
0.01, 0.001	5, 27	3, 58	5, >100	3, >100
0.01, 0.01	5, 18	3, 30	5, 63	3, 56

First no. is for R allele (resistance to Bt broccoli) and second is for B allele (resistance to insecticide).

<sup>a</sup> LTI and HTI are less toxic and highly toxic insecticides.

delaying resistance appear to be somewhat mutually exclusive.

In contrast, parasitism reduces the mean adult diamondback moth density to the low end of the population cycle simulated without parasitoids, but rainfall alone cannot do this (Tables 2 and 4). Parasitism can reduce mean diamondback moth densities by ≈88–99% in the absence of highly toxic insecticide use), whereas rain often makes no difference at all, sometimes only about a 30–50% reduction, and only reaches 90% when highly toxic pesticides knock out parasitoids (Tables 2 and 4). Parasitism rates are 35–40% on average in landscapes with Bt broccoli (Table 4). These values are much lower than the >90% parasitism simulated in the absence of Bt broccoli (Table 2), but they are higher than parasitism obtained with insecticide use (Tables 2 and 4).

**Insecticide Treatments in Refuge.** When an insecticide is applied to the refuge every generation, resistance to Bt broccoli evolves more quickly (Tables 5 and 6) than in the cases without insecticide use (Table 3). This is most obvious in the case of applications of the HTI. Compare “Refuge size” of 0.10 under “Rainfall mortality” in Table 3, for example, where at an initial Bt resistance frequency of 0.001, resistance takes 75 generations without insecticide use and parasitoids, but in Table 5, only six with applications of HTI. Similarly, with a Refuge size of 0.10 (the only one used for Tables 5 and 6) under “No rainfall mortality” in Table 3, again at an initial Bt frequency of 0.001, resistance takes 37 generations without insecticide use and parasitoids, but still only six with applications of HTI (Table 6).

**Table 6.** Number of generations required for the resistance allele frequencies to exceed 50% when an insecticide is applied to the 10% refuge for Bt broccoli and no rainfall mortality occurs in the model

Initial frequencies of R and B	No parasitoids		Parasitoids	
	LTI <sup>a</sup>	HTI <sup>a</sup>	LTI	HTI
0.001, 0.001	23, 10	6, 23	24, 10	6, 22
0.001, 0.01	36, 6	29, 4	41, 6	29, 4
0.01, 0.001	4, 21	3, 23	4, >100	3, 29
0.01, 0.01	4, 15	3, 16	4, >100	3, 16

First no. is for R allele (resistance to Bt broccoli) and second is for B allele (resistance to insecticide).

<sup>a</sup> LTI and HTI are less toxic and highly toxic insecticides.

If resistance to one toxin evolves first, then the area with that toxin can act as a more effective refuge for the second toxin. For example, with both initial allele frequencies equal to 0.001 and under rainfall (Table 5), LTI use is not toxic enough to substantially reduce the effectiveness of the refuge, and resistance to Bt broccoli does not evolve until generation 55, yet resistance to the LTI evolves by generation 10, further prolonging the effectiveness of the refuge because the diamondback moth densities there are no longer suppressed by the insecticide. In contrast for HTI use, its toxicity eliminates the value of the refuge and resistance to Bt broccoli evolves in generation 6, which causes the Bt broccoli plants (at nine times the area) to become a large refuge for HTI-susceptible diamondback moth (Table 5).

If the pest evolves resistance to Bt broccoli quickly in the field, parasitism can contribute to additional delays in the evolution of resistance to insecticides, sometimes dramatically to >100 generations (Tables 5 and 6). With regard to resistance to Bt broccoli, parasitism plays an insignificant role when HTI insecticides are applied to the refuge, as noted above.

Eliminating rainfall mortality of eggs and neonates in the model had little or no effect on the first instance of evolution of resistance, whether it was to Bt broccoli or insecticide (compare Tables 5 and 6). However, lack of rainfall mortality did significantly quicken evolution of resistance to the more durable toxin (either the insecticide or the plant toxin) (Table 6). For example, with both initial allele frequencies equaling 0.001 and an application of HTI, resistance to Bt broccoli evolves in six generations (Tables 5 and 6). This result is the same with or without rainfall mortality. However, resistance to the HTI evolves 40% faster without rainfall mortality (compare Tables 5 and 6). The density independent mortality factor of rainfall reduced all phenotypes equally, but the density of susceptible phenotypes was reduced in refuge eliminating most of the density-dependent population reduction that favors resistant phenotypes in Bt broccoli.

**Effects on Population Densities of Insecticide Use in Refuge.** Insecticide use, either HTI or LTI, in a 10% refuge reduces pest densities significantly over the first 10 generations (Table 4). However, in our model, the consequences of this extra mortality factor are extirpation of the parasitoid population within eight generations (Table 4) and the evolution of resistance to either Bt broccoli or the insecticide (Tables 5 and 6).

**Sensitivity Analysis.** We calculated the model without rainfall mortality to determine the influence of the coefficient that determines the maximum possible parasitism. When the coefficient is increased from the standard 0.95–1.0, both species go extinct within a few generations. This is the case with no toxins in the landscape, only LTI, or with 90% Bt broccoli. For the scenario with no Bt broccoli and LTI applied to all plants, varying the coefficient from 0.80 to 0.99 did not alter the time required for resistance to evolve.

For the scenario with Bt broccoli and a 10% refuge, reducing the coefficient to 0.90 caused a significant delay in evolution of resistance from 75 to >100 generations. Increasing the coefficient to one resulted in resistance evolving in 65 generations, although diamondback moth dropped below  $10^{-12}$  in the third generation. In general, model results are sensitive to the coefficient for maximum possible parasitism and any effects of environment on parasitization.

## Discussion

In the model, parasitism provided the most reliable, long-term management of diamondback moth populations. If we consider an adult density of one diamondback moth per 20 plants as being a reasonable threshold for IPM (<2 young larvae in fields with rainfall), then parasitism by *D. insulare* was the only control that maintained diamondback moth below that threshold in scenarios explored in Tables 2 and 4. The only scenarios in which diamondback moth were not maintained close to or below this threshold involved insecticides that harm the parasitoid. Use of Bt broccoli with a 10% insecticide-free refuge did not reduce the long-term contribution of parasitism to pest control (Table 4).

The results and conclusions of Heimpel et al. (2005) can be used to explain and support those reported here. Heimpel et al. (2005) used an abstract model to demonstrate that high density-independent or density-dependent egg mortality (independent of genotype) delays evolution of resistance. We found the same effect when density-independent rainfall kills pest eggs and neonates. Other kinds of density-independent and genotype-independent mortality can play a significant role in evolution of resistance (Carroll et al. 2012). Heimpel et al. (2005) concluded that for genotype-independent mortality to influence evolution in a landscape consisting of refuge and toxic habitats, it must be followed by density-dependent mortality in a later life stage. Because densities tend to be higher in refuge and because susceptible individuals have higher densities in refuges, indirect selection can occur by equalizing mortality that otherwise would favor the resistant phenotypes in the Bt crop. Our results also demonstrate that density-dependent but genotype-independent mortality caused by natural enemies can delay evolution in patchy landscapes in the same way. Thus, the work presented here and by Heimpel et al. (2005) demonstrate that natural enemies can influence the evolution of pest resistance even when the attacks on pests are neutral with respect to genotype.

Small refuges within Bt broccoli fields can delay evolution of resistance >30 generations if resistance alleles are rare in the pest population (Table 3). However, the effectiveness of these refuges can be compromised by insecticide use. This conclusion regarding the problems with insecticide use supports IRM dogma (Shelton et al. 2000, Onstad 2008) and contradicts the conclusion of Ives and Andow (2002) derived from an abstract model, which likely was only relevant

to scenarios involving species of *Diabrotica* infesting transgenic insecticidal maize.

It is possible that insecticide use could be optimized to prevent evolution of resistance. For example, instead of treating every generation of diamondback moth, insecticide use could be based on an economic-threshold criterion. Or insecticides with different modes of action could be alternated every other generation. We did not model toxin-free refuges for insecticide use (e.g., spraying only 75% of all non-Bt broccoli), but this type of refuge should help delay evolution of resistance to either insecticide. In actual field practice, once resistance evolves to either the Bt or the insecticide, control failures will drive a change in tactics, such that resistance may not evolve as predicted here to the second toxin.

In conclusion, our model results support the idea that Bt crops and biological control can be integrated in IPM and actually synergistically support each other. However, the planting and maintenance of toxin-free refuges are critical to this integration.

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