Journal of Economic Entomology, XX(X), 2017, 1–9
doi: 10.1093/jee/tox323
Research

Density Dependence and Growth Rate: Evolutionary Effects on Resistance Development to Bt (*Bacillus thuringiensis*)

Jeannette C. Martinez,[1,4] Michael A. Caprio,[2] and Nicholas A. Friedenberg[3]

[1]Biopesticides and Pollution Prevention Division, US Environmental Protection Agency, Office of Pesticide Programs, Washington, DC 20460, [2]Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762, [3]Applied Biomathematics, Setauket, NY 11733, and [4]Corresponding author, e-mail: Martinez.Jeannette@epa.gov

Subject Editor: Aaron Gassmann

Received 9 February 2017; Editorial decision 9 November 2017

Abstract

It has long been recognized that pest population dynamics can affect the durability of a pesticide, but dose remains the primary component of insect resistance management (IRM). For transgenic pesticidal traits such as Bt (*Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae)), dose (measured as the mortality of susceptibles caused by a toxin) is a relatively fixed characteristic and often falls below the standard definition of high dose. Hence, it is important to understand how pest population dynamics modify durability and what targets they present for IRM. We used a deterministic model of a generic arthropod pest to examine how timing and strength of density dependence interacted with population growth rate and Bt mortality to affect time to resistance. As in previous studies, durability typically reached a minimum at intermediate doses. However, high population growth rates could eliminate benefits of high dose. The timing of density dependence had a more subtle effect. If density dependence operated simultaneously with Bt mortality, durability was insensitive to its strengths. However, if density dependence was driven by postselection densities, decreasing its strength could increase durability. The strength of density dependence could affect durability of both single traits and pyramids, but its influence depended on the timing of density dependence and size of the refuge. Our findings suggest the utility of a broader definition of high dose, one that incorporates population-dynamic context. That maximum growth rates and timing and strength of interactions causing density dependent mortality can all affect durability, also highlights the need for ecologically integrated approaches to IRM research.

Key words: density dependence, selection, growth rate, Bt, resistance model

Cases of pesticide resistance among agricultural insect pests increased dramatically in the latter half of the 20th century for both chemical pesticides (Mota-Sanchez et al. 2008) and, more recently, transgenic insecticidal traits such as Bt (*Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae)) (Van Rensburg 2007, Storer et al. 2010, Dhurua and Guar 2011, Gassmann et al. 2011, Zang et al. 2011, Farias et al. 2014, Gassmann et al. 2014, Huang et al. 2014). The arsenal of strategies for resistance management is limited and relies to a great degree on dose. The durability of a pesticide, defined as the number of years until resistance, is generally expected to be high at both very low doses, where selection is weak, and very high doses, where selection is so strong that intermediate genotypes remain severely maladapted. Doses between these extremes produce relatively low durability (Comins 1977, Tabashnik and Croft 1982). One obvious consequence of the 'durability valley' is that increasing dose does not always increase durability (Helps et al. 2017), introducing the question of what dose optimizes the combination of control, durability, and cost (Comins 1979).

However, dose cannot be easily manipulated for transgenic pesticidal traits, such as Bt proteins. As such, dose can be understood as a trait's efficacy to cause susceptible mortality; the definition of high dose has been set at 99.99% mortality for single traits (US EPA FIFRA SAP 1998). Real-world efficacy of single Bt traits typically falls short of this ideal (Carrière et al. 2015), potentially placing them low on the durability valley's wall and increasing the relevance of any factors that influence the location of the valley's bottom.

Population dynamics can have a large impact on durability, particularly if the potential for population growth decreases with increasing population density. Studies of the effect of negative density dependence in vital rates suggest variously that it can shorten or extend durability (reviewed in the study by Onstad et al. 2014). This variety of findings owes to the diversity of mechanism, timing, and

form (i.e., strength of competition) that density dependence can take. In general, negative density dependence may extend durability when it acts prior to selection for resistance (Heimpel et al. 2005) or incurs a fitness cost (Gassmann et al. 2012). Conversely, density dependence shortens durability when it acts after selection (Comins 1977, Tabashnik and Croft 1982, Glaum et al. 2012). A higher maximum population growth rate increases this impact by promoting crowding on non-Bt host plants (Ives et al. 2011).

The form of negative density dependence can be classified into three general strengths. Perfect density dependence is a proportional response to changes in density, as might occur in a species that competes for feeding sites. Undercompensation describes less-than-proportional responses to changes in density. Plant defenses have been observed to cause undercompensation (Underwood 2010). Overcompensation describes a more-than-proportional change in population growth rate with changes in density. Overcompensation represents the potential for 'stronger' pest population responses to management actions. Larval cannibalism is one driver of overcompensatory dynamics (Storer et al. 2003).

The strength of negative density dependence is inconsequential if efficacy is high and resistance alleles are rare, such that essentially all larvae on Bt hosts die (Glaum et al. 2012). However, considering a broader range of doses reveals that the form of density dependence can affect the rate of adaptation (Comins 1977, May and Dobson 1986).

Despite extensive empirical research on density dependence in agricultural pests (Hibbard et al. 2010), phytophagous insects more generally (Stiling 1988, Cornell and Hawkins 1995), and broader taxa (Brook and Bradshaw 2006), the degree to which it affects populations on Bt hosts is unknown. Using a simple deterministic model of a panmictic insect population on non-Bt (refuge) and Bt host plants, we examine two gross scenarios of density dependent larval mortality as a function of intra-specific competition. First, larval mortality may be driven by interactions early in the larval life history and act simultaneously with selection. Alternatively, density dependent mortality could be determined by interactions later in the larval life history after selection. This subtle difference alters the relative contribution of density on Bt to total mortality. Under these two models of density dependence, we compare the sensitivity of durability of Bt to dose, maximum population growth rate, and the strength of density dependence.

Materials and Methods

A spatially explicit and deterministic model using a population-level approach to model evolution at up to bi-allelic loci was written in Java version 8.0 (NetBeans IDE 8.1) based on a previous insect model developed by Martinez and Caprio (2016). This model was used to explore the effects of the time and strength of density dependence and the maximum population growth rate (R_{max}) on the time to Bt resistance in a generic arthropod pest. Demographics, movement, and resistance evolution were modeled in a landscape consisting of a matrix of 10 × 10 fields. Each field was assumed to be 50 ha in size and planted to 40 million corn plants (80,000 plants/ha). The spatial structure of the refuge and nonrefuge plants within fields was implicit. The landscape had periodic boundaries (a torus) to eliminate edge effects.

Life History

Our objective was to explore very general interactions between density dependence and the evolutionary process. We, therefore, used a simple life history to simulate a generic arthropod pest. The population had one generation per year; generations did not overlap. Each generation consisted of two time steps, one for a larval stage, and one for the adult stage. All selection affected larvae only via differential survival. Density dependent mortality was modeled as 1) simultaneous, occurring at the same time as selection, and 2) late-acting, where any additional density dependent mortality followed selection prior to adult emergence. Male and female adults dispersed the same amount at the same time. Dispersal took place prior to mating and oviposition. Mating occurred at random and oviposition occurred uniformly in each field. We primarily investigated effects on durability of single Bt toxins and explored several analyses for pyramided traits.

Genetics of Resistance

Genetics reflected a diploid pest with sexual reproduction. Resistance was governed by one autosomal locus per Bt toxin, each having two alleles (*R* for resistance and *S* for susceptibility), producing three possible genotypes (*RR*, *RS*, and *SS*) for the one-gene model and nine possible genotypes for the two-gene model. We assumed resistance was complete. Hence, survival of such individuals exposed to Bt, W_{RR}, was 1.The survival of the heterozygous genotype (W_{RS}) was assumed to be 1.5 times higher than that of the susceptible genotype, W_{SS}, with the constraint that it could not exceed 1. Using a standard equation of dominance (Bourget et al. 2000, equation 1) and substituting $W_{RS} = 1.5\,W_{SS}$ allowed us to solve for dominance, *h*, across efficacies (equation 2).

$$W_{RS} = W_{SS} + h \cdot (W_{RR} - W_{SS}) \tag{1}$$

$$h = \frac{W_{SS}}{2(1 - W_{SS})} \tag{2}$$

For two-locus simulations, we assumed that total survival of Bt selection was the product of survival conferred by each locus and that the egg population was always at Hardy–Weinberg equilibrium.

We modeled an efficacy range from low to high dose (Table 1). Although a cost to resistance has been found in approximately two-thirds of the cases where it was measured (Gassmann et al. 2009), we omitted any cost in the interest of simplicity. Costs typically slow the evolution of resistance (Georghiou and Taylor 1976, Comins 1979, Tabashnik and Croft 1982).

Demographics

The maximum population growth rate, R_{max}, was the product of the reproductive and survivorship potential. R_{max} represents the maximum pest growth factor in the absence of Bt exposure and had the units adults/adults.

Realized population growth rates were reduced from R_{max} by density dependent survival among larvae. We assumed that the population mean survival rate in the absence of Bt was given by the Hassell density dependence function (Hassell 1975).

$$N_{t+1} = N_t R_{max} (1 + aN_t)^{-b}, \tag{3}$$

where *N* is the population density of eggs per plant at time step *t* and *t+1*, the constant *a* is a scaling parameter (units = density^{-1}), and *b* is a unitless exponent that determines the strength with which population growth rate responds to departures from equilibrium population density. With $b < 1$, survival changes slowly with density

Table 1. Generic pest parameters, distributions, and values

Parameters	Values
W_{SS}	0.0001–0.999
W_{RS}	1.5 × W_{SS}
IRAF	0.001
D	1
m	0.30
R_{max}	5, 10, 20, 40, or 80
Initial egg density/field	0.20 × K_{Eggs} for single and 0.05 × K_{Eggs} for dual traits
K_{Eggs}	4.5/plant or 18 million/50 ha*
b (UC)	0.5
b (CC)	1.0
b (SC)	2.0

b, the Hassell density dependent function (equation 3); CC, contest competition; D, diffusivity; IRAF, initial resistance allele frequency; K_{Eggs}, carrying capacity per plant; m, adult dispersal proportion; R_{max}, maximum population growth rate (unitless); SC, scramble competition; UC, undercompensatory competition.

*We assumed 80,000 corn plants/ha; fields were 50 ha; hence, K represents 18 million eggs per field.

(undercompensation). With b = 1, survival changes directly with population density (contest competition). Finally, with b > 1, changes in density bring amplified changes in survival (overcompensation or scramble competition). To ensure models with different values of b were otherwise comparable, we set the scaling constant, a to

$$a = \frac{R_{\max}^{\frac{1}{b}} - 1}{K}, \tag{4}$$

which ensured that population densities were attracted to the carrying capacity, K (units of K = eggs/plant). Although the choice of K has little effect on dynamics in a deterministic model, we chose K = 4.5 eggs/plant. With 80,000 plants/ha in a 50 ha field, a field at carrying capacity had 18 million eggs.

We compared the effects of two distinct modes in which density dependence may operate on a Bt plant. In the first mode we tested, we imagined that selection and density dependence occurred simultaneously. Individuals fated to die from density dependence based on egg density might die from Bt, instead. If Equation 3 is restated as $N_{t+1} = N_t R_{\max} \bar{S}(N_t)$, where $\bar{S}(N_t)$ is the average survival rate taken over all individuals as a function of population density, then simultaneous density dependence is given by

$$\bar{S}(N_t) = \text{Min}\begin{Bmatrix}(1 + aN_t)^{-b} \\ \bar{W}\end{Bmatrix}, \tag{5}$$

where $\bar{W}$ is the average survival of selection by Bt across all individuals. If Bt survival was less than projected from density dependence, then survival was not density dependent. Conversely, if fewer individuals were expect to survive density dependence than selection, an individual's probability of survival was the product of density dependence, $(1/\bar{W})(1 + aN_t)^{-b}$, and its genotype-specific survival rate on Bt. Hence, selection occurred in every generation regardless of density.

In the second mode, density dependence operated on the density of larvae surviving Bt. The population always experienced both kinds of mortality separately. We call this model 'late-acting' because the amount of density dependent survival was determined after Bt mortality occurred.

$$\bar{S}(N_t) = \bar{W}\left(1 + aN_t\bar{W}\right)^{-b}. \tag{6}$$

The two models performed identically in refuge crops (without mortality from Bt toxins, equations (5) and (6) reduce to equation (3)). But the models had different responses to density on Bt plants (Fig. 1). Survival on nonrefuge plants was generally higher under the simultaneous model, density dependence did not occur except under very crowded conditions (note in Fig. 1 that Bt survival and simultaneous density dependence overlap except at high density). When density dependence was weak (b < 1, Fig. 1A), the two models of density dependence could lead to very different survival on Bt plants. Under stronger density dependence (b > 1, Fig. 2B), the two models became more similar. The lack of study of population dynamics in Bt crops means that there is no direct evidence to support one of these models over the other.

Dispersal

Calculation of dispersal followed that in the study by Martinez and Caprio (2016). Interfield movements of dispersing adults were governed by a two-dimensional Gaussian redistribution kernel (Okubo 1980), such that the proportion, v, of individuals moving, for instance, x fields east and y fields north was

$$v(x, y) = \frac{1}{4\pi D} e^{-(x^2+y^2)/4D} \tag{7}$$

where D is the diffusion constant with units of fields^2/time step. For net adult movement in the model's generational time step, D is related to the average total displacement of dispersing adults (d), by $D = 2d^2/\pi$ (Andow et al. 1990). We assumed D = 1, implying an average dispersal distance 1.25 fields per generation. However, we assumed that only a fraction of the population, m, undertook inter-field movements. We set m = 0.3 Postdispersal, we assumed that all adults mixed uniformly within fields via trivial movement (Southwood 1962), leading to random mating and a uniform distribution of eggs on refuge and nonrefuge plants in each field.

Experimental design and analysis

Contributions of Bt and density dependence to resistance evolution

We examined the relative contributions of differential Bt survival and density dependence to the increase in resistance allele frequency over a range of conditions. The direct response to Bt was defined as the change in resistance allele frequency over the whole population measured immediately before and after genotype-specific Bt survival. The contribution of density dependence to resistance evolution was scored as the difference in resistance allele frequency measured immediately after Bt survival and again after density dependence. The use of deterministic simulations with continuous abundance eliminated any genetic drift. The influence of gene flow between fields was eliminated by simulating only a single field. Adult movement between Bt and refuge plants was implicit in the assumption of random mating and a spatially uniform distribution of eggs.

Simulations used single traits with a compliant 20% refuge. The simultaneous density dependence model was used with contest competition. Parameters varied included trait efficacy and maximum population growth rate.

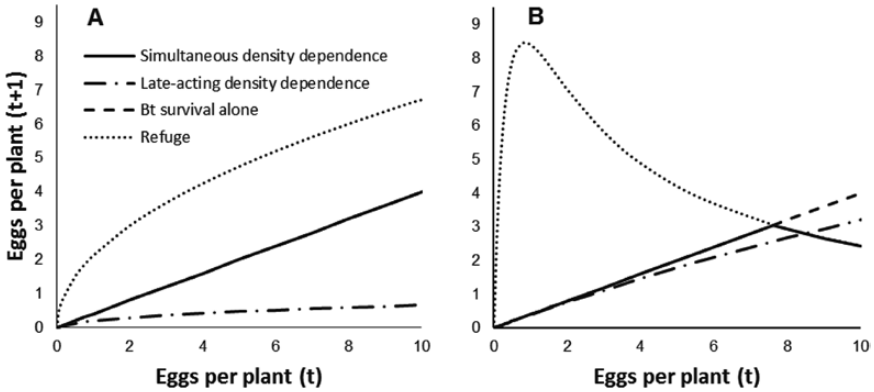

Fig. 1. Population change over one generation when mortality is driven by late-acting density dependence, simultaneous density dependence, Bt mortality alone, or density dependence alone as on refuge plants. (A) Undercompensating density dependence, $b = 0.5$. (B) Scramble competition, $b = 2$. For both panels, $R_{max} = 30$, $W_{ss} = 0.01$, $K = 4.5$ eggs/plant, and initial egg density was 0.2 × K. Heterozygote fitness was 1.5 times that of susceptible fitness ($W_{RS}/W_{SS} = 1.5$).

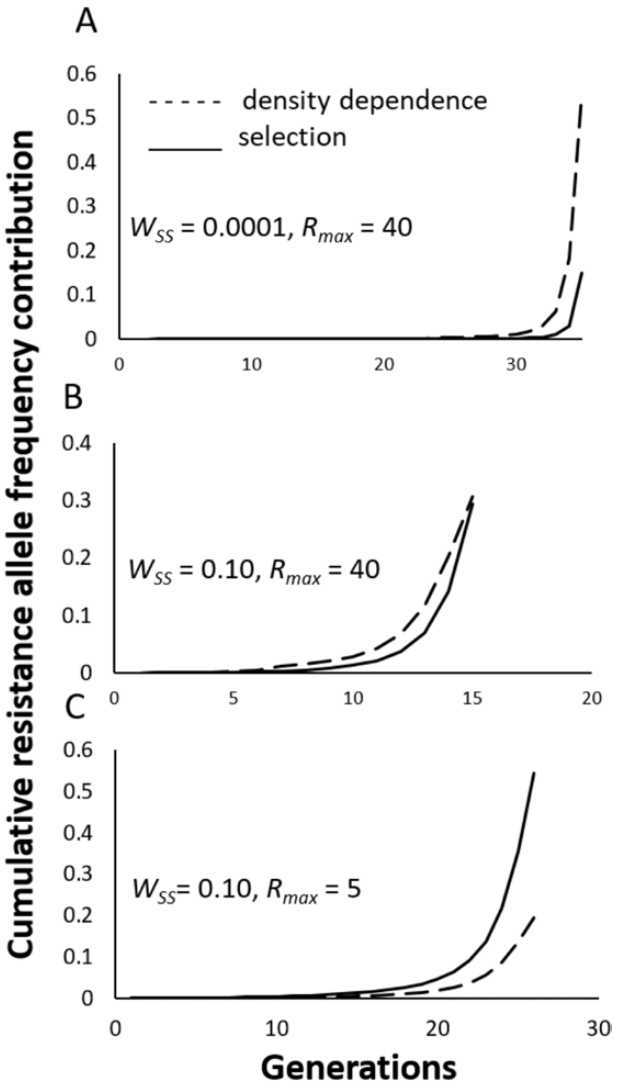

Fig. 2. Contributions of density dependence and selection to the increase in resistance allele frequency under various dose and growth rate assumptions. Results shown for contest competition ($b = 1.0$) with simultaneous model; (A) and (B) HD = 0.9999 and (C) LD = 0.90. Heterozygote fitness was 1.5 times that of susceptible fitness ($W_{RS}/W_{SS} = 1.5$).

The interplay of efficacy with population dynamic variables

A four-way factorial simulation experiment was performed to investigate the response of durability to Bt efficacy, maximum population growth rate, and the strength and timing of density dependence. Bt efficacy ranged from 0.001 to 0.9999 mortality of susceptibles, encompassing a nearly ineffective dose and the EPA standard for high dose. Maximum population growth rate, R_{max}, ranged from 5 to 80 females/female/generation, encompassing slow growth similar to that of western corn rootworm (Szalai et al. 2011) and explosive growth observed in a broad range of arthropod pests (Hassell et al. 1976, Friedenberg et al. 2008, Caprio et al. 2009). The strength of density dependence was varied from undercompensation to scramble competition (b = 0.5, 1, 2). The timing of density dependence was simultaneous with selection or late-acting, as described in Demographics.

Simulations used single traits and a 20% refuge. Grower noncompliance with block refuge requirements was not considered in our simulations, but would lower the durability estimates (Caprio and Glaser 2010, Pan et al. 2011, Martinez and Caprio 2016).

Efficacy and population dynamics with pyramided traits

The time to resistance was measured in fields with pyramided traits and a 5% refuge, consistent with refuge guidelines for pyramids in corn planted in the northern Corn Belt of the United States (US EPA 2010). Durability was measured in a three-way factorial design varying R_{max} (20 and 80), efficacy per trait (survival = 0.001 to 0.2), and the strength of density dependence (undercompensation and overcompensation) using the late-acting model of density dependence. Other parameters followed Table 1.

Quality control in model development

The main code for this generic model was based on a previously tested and published model. However, the current version was re-examined for programming errors and to verify equations. We validated that results were in the expected range (initial resistance allele frequencies, dominance, and Bt mortality) to verify that output behaved as expected. The model was run without selection for 100 generations to assure that the resistance allele frequency did not change. Hardy–Weinberg frequencies for the offspring generation were hand calculated and compared against model output. Exponential population growth was confirmed in the absence of density dependence. In addition, all simulations using late-acting

density dependence were cross-validated with a reproduction of the model in RAMAS IRM, a commercially available modeling tool (Friedenberg et al. 2014).

Results

Evolutionary response to selection and density dependence

The relative importance of the two factors varied with the value of R_{max} and the fitness of the susceptible genotype (dose = 1 – W_{SS}). Density dependence was the main evolutionary force and contributed 0.55 (cumulative) to the rate of adaptation when R_{max} was 40, susceptible dose was 0.9999 (Fig. 2A). The difference in contribution between density dependence and selection was exacerbated when resistance levels increased (generation 30). In the last generation of the simulation, the increase in resistance from density dependent effects was 3.7 times higher than the contribution of selection to resistance (Fig. 2A). The cumulative contribution of density dependence and selection was 80% and 20%, respectively (Fig. 2A). In another high-dose simulation with a lower growth rate, the impact of density dependence was reduced and that of selection elevated (data not shown and similar to Fig. 2B).

The relative importance of density dependence in a selection environment was diminished as a driver of resistance evolution when the dose was intermediate (0.90) with a high pest growth rate (R_{max} = 40) (Fig. 2B). With this dose scenario, the evolutionary response was much less sensitive to population growth rate than in the previous scenario (Fig. 2A), and the cumulative contribution to resistance was 0.3. Contrarily, the impact of selection rose to 0.3 compared with the high-dose scenario. Overall, selection on Bt caused 48% of the 0.59 increase in resistance over 15 generations, whereas density dependence accounted for 52%. The diminished importance of density dependence at an intermediate dose is expected given the reduced dissimilarity of density in refuge and Bt portions of the field.

Finally, when the population growth rate was low (R_{max} = 5) and the dose intermediate (0.90), selection became the dominant evolutionary force after generation 15 (Fig. 2C). Of the 0.73 increase in total resistance allele frequency in 25 generations, selection and density dependence accounted for 74% and 26%, respectively. The diminished importance of density dependence at lower growth rates can be understood as reduced impacts of crowding on fitness in the refuge.

The interplay of efficacy with population dynamic variables

Effects of pest growth rates on the durability of Bt

Durability displayed the typical U-shaped relationship with dose (Tabashnik and Croft 1982) at all but the highest growth rate tested (Fig. 3). In all cases, durability declined precipitously at very low doses (up to about W_{SS} = 0.5). The degree to which durability then increased with further increases in dose depended both on maximum population growth rate and density dependence on Bt plants. The ability to prolong durability with increased dose diminished with increasing R_{max}. Under contest competition, late-acting density dependence produced lower durability at moderate to high dose than did simultaneous density dependence (Fig. 3A vs 3B).

In the case of late-acting density dependence and the highest maximum growth rate tested, R_{max} = 80, increasing the dose never increased durability over the range of doses investigated (Fig. 3A). This particular result highlights two effects of density dependence. First, the fitness of individuals in the refuge is significantly depressed by crowding at high growth rates, and second, released from competition by thorough removal of susceptibles and heterozygotes on traited plants, any fully resistant individuals will realize fitness greater than the refuge population. Our initial frequency of resistance, 0.001, combined with our assumption of initial population density and plants per field, implied that simulations began with four fully resistant eggs, three of them on Bt plants. Additional simulations produced greater sensitivity to dose at high population growth rate when resistance alleles were rare enough that less than one fully resistant egg was expected in the initial population.

Effects of strength of density dependence on the durability of Bt

The strength of density dependence only affected durability in the late-acting density dependence model (Fig. 4). Undercompensation increased durability. For our undercompensation scenario of b = 0.5, durability relative to contest competition increased with increasing dose. This increase occurred at a lower dose when maximum population growth rate was higher. At high dose, undercompensation extended durability by 41, 61, and 55% at R_{max} = 5, 20, and 40, respectively. Scramble competition decreased durability in the late-acting density dependence model. For our scramble competition with b = 2, durability relative to contest competition decreased with increasing dose. As with the effect of undercompensation, scramble

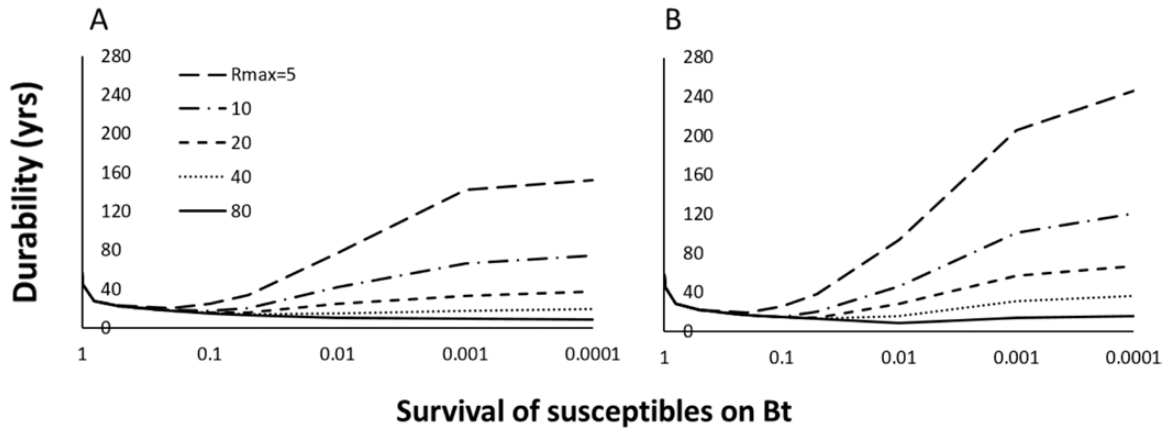

Fig. 3. The effect of growth rate R_{max} on durability of Bt with (A) late-acting or (B) simultaneous density dependence for fitness ranging from 1 to 0.0001 (note that x-axis uses a Log scale of [1-dose]). Growth rates: R_{max} = 5, 10, 20, 40, and 80; heterozygote fitness was 1.5 times that of susceptible fitness (W_{RS}/W_{SS} = 1.5).

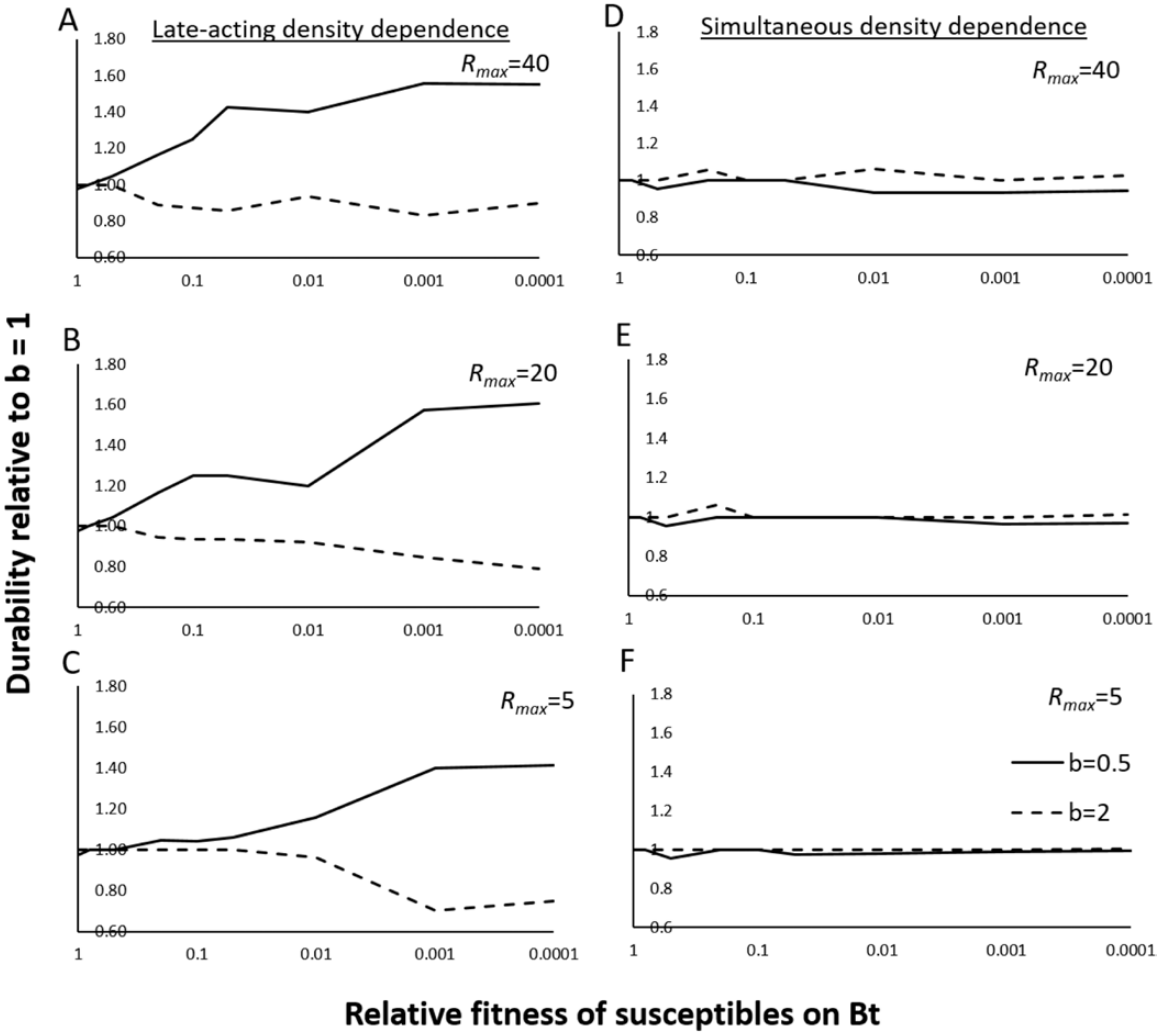

Fig. 4. Strength of density dependence expressed relative to contest competition for fitness ranging from 1 to 0.0001 (note that x-axis uses a Log scale of [1-dose]) when the pest exhibited undercompensating or scramble competition with different maximum growth rates. R_{max} = 5, 20, and 40; undercompensatory competition, b = 0.5; SC, b = 2.0. Heterozygote fitness was 1.5 times that of susceptible fitness (W_{RS}/W_{SS} = 1.5). (A)–(C) Late-acting model; (D)–(F) simultaneous model.

competition's effect occurred at a lower dose when maximum population growth rate was higher. At high dose, scramble competition shortened durability by 25, 21, and 10% at R_{max} = 5, 20, and 40, respectively, suggesting that the effect of overcompensatory density dependence diminishes with increases in maximum population growth rate.

Efficacy and population dynamics with pyramided traits

As with single Bt traits, the durability of pyramids was a U-shaped function of dose; time to resistance was shortest for intermediate trait efficacy (W_{SS} = 0.2–0.8). Higher maximum growth rates decreased durability of pyramids for W_{SS} < 0.50 per trait (Table 2). For instance, pests with a 20% refuge and scramble competition feeding on a pyramid with susceptible survival of 0.05 per trait had a 72% shorter time to resistance with a maximum population growth of 80 rather than 20. This effect of size held true for 5% refuges, as well (Table 2).

The effect of the strength of density dependence on durability depended on maximum growth rate, trait efficacy, and refuge size. For instance, the strength of density dependence had almost no effect on durability of a pyramid with susceptible survival of 0.001 per trait regardless of refuge size or maximum growth rate (Table 2). However, the durability of a trait with more moderate efficacy of W_{SS} = 0.1 was sensitive to the strength of density dependence when maximum population growth rate was high and the refuge was large, falling 47% as b was increased from 0.5 to 2 under an R_{max} of 80 and a 20% refuge (Table 2).

Discussion

Our simulations suggest that the influence of population dynamics on resistance evolution can be as substantial as that of dose for both single traits and pyramids. Of the factors investigated, maximum population growth rate had the greatest leverage on durability at any given dose and modulated the relative contributions of direct and indirect selection exerted by Bt toxins. These results corroborate prior studies of the role of density dependence in resistance evolution (reviewed in Onstad et al. 2014). Additionally, we found that the strength of density dependence had a nontrivial effect on durability, but the presence of these effects depended on refuge size and how density dependence worked on Bt plants. With dose a central focus

Table 2. Durability (years) of a pyramid as a function of maximum population growth rate, strength of competition, and trait efficacy under late-acting density dependence

			Efficacy per trait				
Refuge	R_{max}	Strength	0.2	0.1	0.05	0.01	0.001
20%	20	Undercompensation	30	59	170	2,178	47,843
		Scramble competition	23	56	169	2,179	47,844
20%	80	Undercompensation	28	36	61	554	11,953
		Scramble competition	16	19	47	548	11,952
5%		Undercompensation	17	19	37	454	10,055
		Scramble competition	15	17	40	462	10,063

Efficacy of the two traits was equal. Resistance was conferred by a single bi-allelic locus per trait. The relative fitness of single heterozygotes was 1.5 for each trait. Undercompensation, $b = 0.5$. Scramble competition, $b = 2$. Simulations began with egg abundance at $0.05 \times K_{Eggs}$.

in the design of transgenic pesticidal hybrids and a major component of the high-dose/refuge strategy, it may be productive to broaden the concept of high dose to incorporate population context.

We observed the largest effects of density dependence at high maximum population growth rates, where crowding in the refuge and the high reproductive potential of survivors on traited plants led to rapid resistance evolution even for true high dose traits. The range of growth rates we examined has real-world relevance. Some insect pests have dramatic growth potential. For example, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) on potato may increase its abundance 75-fold at low density (Hassell et al. 1976). Other major pests have far more moderate growth rates, such as *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), which may increase less than 5-fold per generation (Szalai et al. 2011). The same hypothetical trait would likely lead to radically different durability in these two species, even if it met the standard definition of high dose.

Previous studies have noted the influence of the strength of density dependence on resistance evolution, finding that undercompensation generally gives greater durability than scramble competition (Comins 1977, May and Dobson 1986). We observed that this influence may not always exist. If the reduction of population density by Bt mutes density dependent processes on nonrefuge plants, then the strength of density dependence does not affect the rate of resistance evolution. Our model of simultaneous density dependence is only one example of how survival on Bt plants could be insensitive to population density. Another example would be threshold density dependence, in which survival is constant in uncrowded conditions (Hibbard et al. 2010). Because density dependence can arise from a number of different ecological drivers, including competitors, host plant defenses, predators, and pathogens and at different points in the life history (Stiling 1988, Cornell and Hawkins 1995), it is possible that Bt mortality targeting, for instance, early larvae could expose density dependent processes among late-instar larvae that were previously obscured. Our results reveal a research need to determine the timing and mechanisms of pest density dependence on Bt host plants, especially when the intrinsic growth rate is high.

Pyramided traits displayed less sensitivity to the details of population dynamics. While maximum population growth rate was still important, the strength of density dependence only affected pyramid durability in the larger refuge size we examined. In the United States, guidelines allow for a 5% refuge in pyramid corn with the exception of the southern US where a 20% block refuge is required (US EPA 2010), which appear to be so small that severe crowding would not occur except at extreme population growth rates. Pyramids also nullified the difference in durability between undercompensation and scramble competition when traits had higher doses. In the absence of an effect of the strength of density dependence, our study suggests that insect resistance management does not need to consider the details of density dependence on nonrefuge plants for pyramided traits.

Given the importance of density dependent population growth, it is likely that innovations in IRM will arise from a greater empirical understanding of population dynamics in agricultural pests. The standard for high dose, 0.01% survival of susceptibles, was codified in five recommendations provided by a scientific advisory panel to the EPA (US EPA FIFRA SAP 1998). While this level of efficacy proved durable in most scenarios we explored, real-world transgenic events do not appear to achieve formal high dose performance often (Carrière et al. 2015). Likewise, though we found in additional simulations (not shown) that lower initial frequency of resistance reduced the sensitivity of durability to maximum population growth rate, IRM cannot rely on hoping for ideal conditions of susceptibility. Pyramids help to address the problem of resistance allele frequency by making full heterozygotes or resistance genotypes exceedingly rare (Ives et al. 2011). However, this theoretical benefit accrues only if resistance to the component traits is not already common.

Population dynamics present a difficult challenge for resistance management of Bt. A pest's population growth rate can vary geographically (Chen et al. 2014) and temporally (Szalai et al. 2011), due to both random events and measurable factors such as temperature (Philipp and Watson 1971). Furthermore, the effects of diet on somatic growth and fecundity (Fleischer and Gaylor 1988, Chen and Parajulee 2010, Jha et al. 2012) can alter the population growth potential of successive cohorts utilizing different hosts (Caprio et al. 2009). The mechanism of density dependence may differ on different hosts, as well (Stiling 1988), leading to different strength and timing relative to selection. A sign of hope for the integration of predictive population dynamic models into IRM can be found in a meta-analysis of several *D. virgifera virgifera* studies (Hibbard et al. 2010), which showed a consistent pattern of density dependent immature survival despite differences in maximum survival among studies.

Previous studies have suggested that dose could be tailored to the agricultural context. Tabashnik and Croft (1982) proposed that low-dose pesticides should be preferred over intermediate and high-dose treatments in the absence of a refuge. A low-dose Bt product may not be an attractive option for growers, however, because of greater risk of crop damage or the need for additional chemical spraying to control pest densities. For traditional sprayed pesticides, the lowest dose to achieve target pest densities is optimal in terms of cost, control, and resistance management (Comins 1979). But the dose of a Bt hybrid is fixed from the grower's perspective and cannot be manipulated to respond to pest outbreaks. Transgenic pesticidal traits are prophylactic strategies by definition and meant to

provide control at all possible pest population levels, making high efficacy essential.

In some cases, the same insect in different crops might experience very different regimes of density dependent mortality. For example, in conventional cotton, Heliothines (*Helicoverpa zea* (Boddie) [Lepidoptera: Noctuidae] and *Heliothis virescens* (Fabricius) [Lepidoptera: Noctuidae]) are treated at levels as low as 4 larvae per 100 plants, thereby avoiding most density dependent mortality. In contrast, *H. zea* is unlikely to be sprayed on conventional corn. If multiple larvae infest the same ear, cannibalism may result (Storer et al. 2001), usually with only one to two survivors. This extensive density dependent mortality could hasten the evolution of resistance in *H. zea* to transgenic corn, leading to resistance in cotton hybrids with similar traits.

If high levels of density dependent mortality threaten resistance management strategies for some species, then one potential answer would be to reduce the net growth rate of the species, otherwise known as IPM or integrated pest management. Strategies such as crop rotation, trap crops, mating disruption, natural enemy releases, sterile male release, etc., as long as they impact populations in Bt fields and refuges equally, would reduce the net growth rate of targeted species and potentially extend durability of transgenic crops (Carrière and Tabashnik 2001, Crowder et al. 2005, Onstad 2008, Onstad et al. 2011). Even insecticidal oversprays would act to lower the net growth rate of the species, though they introduce cost, environmental concerns, and struggles with resistance to chemistry that plant-incorporated-protectants were meant to avoid. Finally, control of refuge densities, even if its goal is to increase the fitness of susceptible pests, may appeal to growers concerned that refuge plants are a sacrificial investment. Hence, the tenets of IPM are still relevant in an age of transgenic crops) (Onstad et al. 2011).

Acknowledgments

We thank J. Schneider for lending his ecological and mathematical expertise and R. Luttrell, F. Musser, and A. Catchot for their review of the original work published in the dissertation. J. Leahy and A. Reynolds provided insightful comments on an earlier draft of the manuscript. We also thank two anonymous reviewers for their comments. Opinions, conclusions, and recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the US Environmental Protection Agency.

References Cited

Andow, D. A., P. M. Karieva, S. A. Levin, and A. Okubo. 1990. Spread of invading organisms. Landscape Ecol. 4: 177–188.

Bourget, D., A. Genissel, and M. Raymond. 2000. Insecticide resistance and dominance levels. J. Econ. Entomol. 93: 1588–1595.

Brook, B. W., and C. J. A. Bradshaw. 2006. Strength of evidence for density dependence in abundance time series of 1198 species. Ecol. 87:1445–1451.

Caprio, M. A., C. D. Parker, and J. C. Schneider. 2009. Future fitness of female insect pests in temporally stable and unstable habitats and its impacts on habitat utility as refuges for insect resistance management. J. Insect Sci. 9: 1–10.

Caprio, M. A. and J. A. Glaser. 2010. Simulation models evaluation of pest resistance development to refuge in the bag concepts related to Pioneer submission. Letter report from the US EPA ORD, Sustainable Technology Division, National Risk Management Research Laboratory, Cincinnati, OH, to the US EPA, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division, Washington DC. Last accessed on April 12, 2016. http://nepis.epa.gov/Adobe/PDF/P100EBX2.pdf

Carrière, Y., and B. E. Tabashnik. 2001. Reversing insect adaptation to transgenic insecticidal plants. Proc. R. Soc. B. Biol. Sci. 268: 1475–1480.

Carrière, Y., N. Crickmore, and B. E. Tabashnik. 2015. Optimizing pyramided transgenic Bt crops for sustainable pest management. Nat. Biotech. 33: 161–168.

Carrière, Y., J. A. Fabrick, and B. E. Tabashnik. 2016. Can pyramids and seed mixtures delay resistance to Bt crops? Trends Biotechnol. 34: 291–302.

Chen, C., and M. N. Parajulee. 2010. Development and population growth of Lygus hesperus on selected weed hosts, artificial diet and cotton in the laboratory. J. Econ. Entomol. 103: 2009–2018.

Chen, J., A. Alyokhin, D. Mota-Sanchez, M. Baker, and M. Whalon. 2014. Variation in fitness among geographically isolated Colorado potato beetle (Coleoptera: Chrysomelidae) populations. Ann. Entomol. Soc. Am. 107: 128–135.

Comins, H. N. 1977. The development of insecticide resistance in the presence of migration. J. Theor. Biol. 64: 177–197.

Comins, H. N. 1979. Analytic methods for the management of pesticide resistance. J. Theor. Biol. 77: 171–188.

Cornell, H. V., and B. A. Hawkins. 1995. Survival patterns and mortality sources of herbivores insects: some demographic trends. Am. Nat. 145: 563–593.

Crowder, D. W. D. W. Onstad, M. E. Gray, P. D. Mitchell, J. L. Spencer, and R. J. Brazee. 2005. Transgenic corn for the control of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98: 961–975.

Dhurua, S., and G. T. Gujar. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora* gossypiella (Saunders) (Lepidoptera: Gelechiidae), from India. Society of Chemical Industry, http://onlinelibrary.wiley.com/doi/10.1002/ps.2127/pdf (accessed 27 September 2017).

Farias, J. R., D. A. Andow, R. J. Horikoshi, R. J. Sorgatto, P. Fresia, A. C. dos Santos, and C. Omoto. 2014. Field evolved resistance to Cry1F maize by Spodoptera frugiperda (Lepidoptera: Noctuidae) in Brazil. Crop Protection 64: 150–158.

Fleischer, S. J., and M. J. Gaylor. 1988. Lygus lineolaris (Heteroptera: Miridae) population dynamics: nymphal development, life tables, and Leslie matrices on selected weeds and cotton. Environ. Entomol. 17: 246–253.

Friedenberg, N. A., K. T. Shoemaker, and W. Root. 2014. RAMAS IRM version 2: software for risk-based durability assessment. Applied Biomathematics, Setauket, NY.

Friedenberg, N. A., S. Sarkar, N. Kouchoukos, R. F. Billings, and M. P. Ayres. 2008. Temperature extremes, density dependence, and southern pine beetle (Coleoptera: Curculionidaea) population dynamics in east Texas. Environ. Entomol. 37: 650–659.

Gassmann, A. J. 2012. Field-evolved resistance to Bt maize by western corn rootworm: predictions from the laboratory and effects in the field. J. Invert. Pathol. 110: 287–293.

Gassmann, A. J., Y. Carrière, and B. E. Tabashnik. 2009. Fitness costs of insect resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 54: 147–163.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLoS One 6: e22629.

Gassmann, A. J., J. L. Petzold-Maxwell, E. H. Clifton, M. W. Dunbar, A. M. Hoffmann, D. A. Ingber, and R. S. Keweshan. 2014. Field evolved resistance bywestern corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. Proc. Natl. Acad. Sci. USA.. 111: 5141–5146.

Georghiou, G. P., and C. E. Taylor. 1977. Genetic and biological influences in the evolution of insecticide resistance. J. Econ. Entomol. 70: 319–323

Glaum, P. R., A. R. Ives, and D. A. Andow. 2012. Contamination and management of resistance evolution to high-dose transgenic insecticidal crops. Theor. Ecol. 5: 195–209.

Hassell, M. P. 1975. Density-dependence in single species populations. J. Anim. Ecol. 44: 283–295.

Hassell, M. P., J. H. Lawton, and R. M. May. 1976. Patterns of dynamical behaviour in single-species populations. J. Anim. Ecol. 45: 471–486.

Heimpel, G., C. Neuhauser, and D. A. Andow. 2005. Natural enemies and the evolution of resistance to transgenic insecticidal crops by pest insects: the role of egg mortality. Envior. Entomol. 35: 512–526.

Helps, J. C., N. D. Paveley, and F. van den Bosch. 2017. Identifying circumstances under which high insecticide dose increases or decreases resistance selection. J. Theor. Biol. 428: 153–167.

Hibbard, B. E., L. N. Meihls, M. R. Ellersieck, and D. W. Onstad. 2010. Density-dependent and density-independent mortality of the western corn rootworm: impact on dose calculations of rootworm-resistant Bt corn. J. Econ. Entomol. 103: 77–84.

Huang, F., J. A. Qureshi, R. L. Meagher Jr., D. D. Reisig, G. P. Head, D. A. Andow, X. Ni, D. Kerns, G. D. Buntin, Y. Niu, F. Yang, and V. Dangal. 2014. Cry1F resistance in fall armyworm Spodoptera frugiperda: single gene vs. pyramided Bt maize. PLoS One 9: e112958.

Ives, A. R., P. R. Glaum, N. L. Ziebarth, and D. A. Andow. 2011. The evolution of resistance to two-toxin pyramid transgenic crops. Ecol. Appl. 21: 503–515.

Jha, R. K., H. Chi, L. C. Tang. 2012. A comparison of artificial diet and hybrid sweet corn for the rearing of Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) based on life-table characteristics. Environ. Entomol. 41: 30–39.

Liu, N., M. Li, Y. Gong, F. Liu, and T. Li. 2015. Cytochrome P450s—their expression, regulation, and role in insecticide resistance. Pestic. Biochem. Physiol. 120: 77–81.

Martinez, J. C. and M. A. Caprio. 2016. IPM use with the deployment of a non-high dose Bt pyramid and mitigation of resistance for western corn rootworm (*Diabrotica virgifera virgifera*). Environ. Entomol. 45: 747–761.

May, R. M., and A. P. Dobson. 1986. Population dynamics and the rate of evolution of pesticide resistance, pp. 170–193. *In* National Research Council. Pesticide Resistance: Strategies and Tactics for Management. The National Academies Press, Washington, DC.

Mota-Sanchez, D., M. E. Whalon, R. M. Hollingworth, and Q. Xue. 2008. Documentation of pesticide resistance in arthropods, p. 32–40. In M. E. Whalon, (ed.), Global pesticide resistance in arthropds. CABI Publishing, Trowbridge, UK.

Okubo, A. 1980. Diffusion and ecological problems: mathematical models. Springer Verlag, New York. 254 pp.

Onstad, D. W. 2008. Insect resistance management: biology, economics, and predictions. Academic, San Diego, CA.

Onstad, D. W., P. D. Mitchell, T. M. Hurley, J. G. Lundgren, R. P. Porter, C. H. Krupke, J. L. Spencer, C. D. DiFonzo, T. S. Bauté, R. L. Helmich, et al. 2011. Seeds of change: corn seed mixtures for resistance management and integrated pest management. J. Econ. Entomol. 104: 343–352.

Onstad, D. W., A. M. Shelton, and J. L. Flexner. 2014. Insect resistance, natural enemies, and density dependent processes, pp. 403–420. In D. W. Onstad, (ed.), Insect resistance management, 2nd ed. Academic Press, New York, NY.

Pan, Z., D. W. Onstad, T. M. Nowatzki, B. H. Stanley, L. J. Meinke, and J. L. Flexner. 2011. Western corn rootworm (Coleoptera: Chrysomelidae) dispersal and adaptation to single-toxin transgenic corn deployed with block or blended refuge. Env. Entomol. 40(4): 964–978.

Pedra, J. H., R. A. Festucci-Buselli, W. Sun, W. M. Muir, M. E. Scharf, and B. R. Pittendrigh. 2005. Profiling of abundant proteins associated with dichlorodiphenyltrichloroethane resistance in *Drosophila melanogaster*. Proteomics 5: 258–269.

Philipp, J. S. and T. S. Watson. 1971. Influence of temperature on population growth of the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). Ann. Entomol. Soc. Am. 64: 334–340.

Reierson, D., and M. Rust. 1996. Increased dosage (saturation) to overcome poor control associated with insecticide resistance in urban insect pests. *In* Proceedings of the second international conference on Urban Pests, Edinburgh. 1. http://www.icup.org.uk/report/ICUP751.pdf (accessed 30 November 2017).

Szalai, M., J. P. Komaromi, R. Bazok, J. I. Barcic, J. Kiss, and S. Toepfer. 2011. Generational growth rate estimates of *Diabrotica virgifera virgifera* populations (Coleoptera: Chrysomelidae). J. Pest Sci. 84: 133–142.

Southwood, T. R. E. 1962. Migration of terrestrial arthropods in relation to habitat. Biol. Rev. 37: 171–214.

Stiling, P. 1988. Density-dependent processes and key factors in insect populations. J. Anim. Ecol. 57: 581–593.

Storer, N. P., J. W. Van Duyn, and G. G. Kennedy. 2001. Life history traits of *Helicoverpa zea* (Lepidoptera: Noctuidae) on non-Bt and Bt transgenic corn hybrids in eastern North Carolina. J. Econ. Entomol. 94: 1268–1279.

Storer, N. P., J. W. van Duyn, and G. G. Kennedy. 2003. Life history traits of Helicoverpa zea (Lepidoptera: Noctuidae) on non-Bt and Bt transgenic corn hybrids in Eastern North Carolina. J. Econ. Entomol. 94: 1268–1279.

Storer, N. P., J. M. Babcock, M. Schelnz, T. Meade, G. D. Thompson, J. W. Bing, and R. M. Huckaba. 2010. Discovery and characterization of field resistance to Bt maize: Spodoptera frugiperda (Lepidoptera: Noctuidae) in Puerto Rico. J Econ. Entomol. 103: 1031–1038.

Tabashnik, B. E., and B. A. Croft. 1982. Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. Environ. Entomol. 11: 1137–1144.

Underwood, N. 2010. Density dependence in insect performance within individual plants: induced resistance to Spodoptera exigua in tomato. Oikos 119: 1993–1999.

US EPA [United States Environmental Protection Agency]. 2010. Terms and conditions for Bt corn registration. Office of Pesticide Programs, September 30, 2010. Last accessed on November 16, 2016. https://www3.epa.gov/pesticides/chem_search/reg_actions/pip/bt-corn-terms-conditions.pdf

US EPA FIFRA SAP [United States Environmental Protection Agency Federal Insecticide, Fungicide and Rodenticide Act Scientific Advisory Panel]. 1998. Transmittal of Final Report of the FIFRA Scientific Advisory Panel Subpanel on Bacillus thuringiensis (Bt) Plant-Pesticides and resistance management. Meeting held on February 9 and 10, 1998. Memo from Dr. E. Milewsiki (DFO) to L. Goldman (AA Office of Prevention, Pesticides and Pollution Prevention). Last accessed on July 17, 2017. https://archive.epa.gov/scipoly/sap/meetings/web/pdf/finalfeb.pdf

Van Rensburg, J. B. J. 2007. First report of field resistance by the stem borer, Busseola fusca (Fuller) to transgenic maize. S Afr J Plant Soil 24: 147–151.

Zhang, H., W. Yin, J. Zhao, L. Jin, Y. Yang, S. Wu, B. E. Tabashnik, and Y. Wu. 2011. Early warning of cotton bollworm resistance associated with intensive planting of Bt cotton in China. PLoS One 6: 1–8.