Bt RESISTANCE UNDER CONVENTIONAL INSECTICIDE USE

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Abstract

Theoretical models and available field and laboratory data suggest that the development of resistance in Louisiana bollworms (H. zea) and budworms (H. virescens) to Bacillus thuringiensis (Bt) and pyrethroids is influenced by assumptions made regarding the use of pyrethroids on refuge acres. We show that the use of untreated refuge regenerates susceptibility to pyrethroids in both insects, leading to a toxin-mixture effect that significantly reduces the rate of Bt resistance development relative to the treated-refuge case.

Introduction

The majority of genetic and entomologic studies of insecticide resistance have examined single-insect single-insecticide models, as in Georghiou and Taylor (1977a, 1977b). Note that Dawson (1970), Ford (1971), Cavener and Clegg (1981), Chevillon et al. (1995), Taylor et al. (1983), and Tabashnik and Croft (1985) support the accuracy of the so-called, single-locus genetic model and its central tenet, the Hardy-Weinberg equilibrium principle. Since cotton producers routinely use multiple insecticides and insecticide mixtures to manage multiple insect pests, and since Georghiou (1983), Curtis (1985), Mani (1986), and Taylor (1986) suggest that toxin mixtures can reduce the rate of resistance development relative to the single-insecticide case, the single-locus model may not be well suited for examining resistance development under realistic cotton production settings.

Two-locus genetic models, therefore, are used to examine bollworm and budworm resistance development to pyrethroids and Bt under various assumptions regarding pyrethroid use (Futuyma, 1986; Gould, 1986). Model parameters are estimated using Louisiana, state-level field data on pyrethroid resistance, and available field and laboratory data on Bt resistance. Since model parameters are estimated using Louisiana data, implications of the analysis apply strictly to Louisiana bollworms and budworms.

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Model and Assumptions

We summarize the main features of the genetic model in this section. See Livingston (1999) for a complete presentation. The two-locus model of resistance for each pest is taken, with minor modification, from Futuyma (1986). Pyrethroid resistance is conferred to insects at locus one, and Bt resistance is conferred to insects at locus two. Let x and X denote the alleles that confer resistance and susceptibility to pyrethroids at locus one, respectively; and let y and Y denote the alleles that confer resistance and susceptibility to Bt at locus two. Table 1 lists the nine genotypes, their constant probabilities of surviving to adulthood and reproducing when facing Bt cotton and pyrethroids, and their constant probabilities of surviving to adulthood and reproducing when facing pyrethroids used singly on non-Bt refuge. The parameter ϵ is set to zero when non-Bt refuge is not treated with pyrethroids.

Fitness costs associated with homozygous pyrethroid-resistant genotypes, α, and homozygous Bt-resistant genotypes, β, are included. Briefly, γ denotes the constant mortality rate of Bt-susceptible genotypes, ϵ, denotes the constant mortality rate of pyrethroid-susceptible genotypes, and δ_b and δ_p denote the degrees of recessiveness of the inherited Bt- and pyrethroid-resistance traits, respectively. Values close to one indicate that resistance is inherited as a recessive trait, and values close to zero indicate that resistance is inherited as a dominant trait.

We assume that: (i) resistant and susceptible insects survive insecticides at different rates; (ii) alleles do not mutate; (iii) there is no migration of insects into or out of the population; (iv) there are no random events that cause some insects to pass on more of their genes than others; (v) mating is random, and reproduction is sexual; (vi) linkage equilibrium; and (vii) insects are equally distributed across Bt and non-Bt acres. Assumption (i) specifies selection as the mechanism of evolution, and assumptions (ii) through (v) specify selection as the only possible mechanism of evolution. It is not clear if the assumption of linkage equilibrium holds for bollworms and budworms. However, it is a reasonable assumption when loci are on two separate chromosomes, or on the same chromosome but far apart. Further, Mani (1986) showed that linkage equilibrium reduced the dampening effect toxin mixtures have on the rate of resistance development. Therefore, we model toxin-mixture effects conservatively.

We assume that bollworms and budworms go through three generations per cotton growing season and two generations between growing seasons in Louisiana. We assume that 3.5% of bollworms and budworms survive the winter generation and then experience one subsequent generation on natural hosts prior to the availability of cultivated hosts (Stadelbacher and Martin, 1980). We assume that bollworms experience one complete generation in corn, after their first generation on natural hosts, before moving into cotton. We assume that budworms move directly into cotton after their first generation on natural hosts. Bollworms and budworms, therefore, are selected for Bt and pyrethroid resistance during two and three generations per year, respectively.

Because data are not available, genetic-model parameters associated with Bt resistance are specified exogenously. See Livingston (1999) for a thorough sensitivity analysis of alternative parameter values on resistance. Note that estimates of pyrethroid-resistance parameters do not vary with Bt-resistance parameter values. Fitness costs of Bt-resistant homozygotes are set to zero for both insects (Gould and Anderson, 1991). Bt resistance is inherited as a completely recessive trait in both insects (Sims and Stone, 1991). Mortality rates for Bt-susceptible bollworms and budworms are 0.60 and 0.95, respectively (Hardee et al., 1997; Lambert et al., 1998). The initial, budworm Bt resistance-allele frequency is 0.0015 (Gould et al., 1997). In terms of LC50 values budworms are approximately nine times more susceptible to Bt than bollworms (Hardee et al., 1997). Since an estimate of the initial, Bt resistance-allele frequency is unavailable for bollworms, we multiply the budworm estimate (0.0015) by nine, setting it to 0.0135.

Data and Estimation

The inputs for each genetic model are the proportion of Bt cotton planted in Louisiana, and pyrethroid and Bt resistance-allele frequencies at the beginning of the growing season. The outputs of each genetic model are the pyrethroid and Bt resistance-allele frequencies at the end of each insect generation over the entire year, larval survival rates facing Bt cotton and pyrethroids averaged over the growing season, and larval survival rates on non-Bt cotton averaged over the growing season. We assume perfect information over the equations of motion for unobservable resistance-allele frequencies, treating them as latent variables in the estimation problems.

Bagwell et al. (1999) and Plapp and Campanhola (1986) provide pyrethroid survival rates using the Adult Vial Test (AVT) for adult male bollworm and budworm moths collected in Louisiana over the 1987 through 1998 growing seasons. We use Plapp and Campanhola (1986) for the 1987 bollworm observation. Since adult moth survival rates can be used as proxies for larval survival rates, these data are used as observations on the dependent variable in the estimation problems (Gage and Hatfield, 1989). We use budworm survival rates facing ten micrograms of cypermethrin per vial and bollworm survival rates facing five micrograms of cypermethrin per vial, averaged over all Louisiana parishes sampled and over each growing season, for a total of 12 observations per insect.

Foliar Bt insecticides have been in use for over 50 years (Cannon, 1993). Since Gould et al. (1992) showed that laboratory selection of the budworm with a popular Bt toxin conferred broad cross-resistance to other Bt toxin varieties, we account for the use of foliar Bt insecticides. In particular, observations on the proportion of cotton acres sprayed with foliar Bt insecticides during the 1993, 1994, and 1995 Louisiana crop years are used as proxies for the proportion of Bt cotton planted (United States Department of Agriculture, 1994, 1995, and 1996). Observations are available on the proportion of Bt cotton planted during the 1996, 1997, and 1998 Louisiana crops years (Monsanto, 1998; Williams, 1997, 1998, and 1999). We average both series due to minor discrepancies. For the years 1987 to 1992, the proportion of Bt cotton planted in Louisiana is zero.

Since parameters enter the two-locus genetic model nonlinearly, we use unconstrained Gauss-Newton with line search to obtain the least-squares parameter estimates (Fletcher, 1987). A change of variables is used to account for the fact that all parameters are constrained to the unit interval. Table 2 provides the estimation results. The coefficients of determination in the non-linear regressions are not constrained to the unit interval, indicating that good overall fits were obtained. Except for the estimate of the initial, pyrethroid resistance-allele frequency, all of the budworm parameter estimates are statistically significant. All of the bollworm parameter estimates are statistically insignificant, except for the estimate of the susceptible-genotype, pyrethroid mortality rate. Inspection of the estimated covariance matrices indicates that budworm parameter estimates do not vary appreciably with the estimate of the initial allele frequency; whereas the insignificant bollworm parameter estimates do. Furthermore, the budworm dependent variable observations exhibit almost ten times more variation than the bollworm observations. The lack of variation in the dependent variable coupled with significant initial-allele-frequency covariance may have contributed to the insignificance of the bollworm estimates.

The levels of the parameter estimates, however, agree with the general finding that budworms are less susceptible to pyrethroids than bollworms. The estimate of the susceptible-genotype mortality rate for budworms (0.86) is less than the bollworm estimate (0.95). Although insignificant, the estimate of the initial pyrethroid resistance-allele frequency for budworms is almost ten times larger than the bollworm estimate. Similarly, the estimate of the fitness cost of budworms homozygous for pyrethroid resistance (0.51) is less than the bollworm estimate (0.57). Fitness-cost estimates of this magnitude are rare but, apparently, have been reported elsewhere in studies of different insects (Gould, 1999). The budworm estimate of the degree of recessiveness of the inherited pyrethroid-resistance trait (0.83) agrees with Roush and Luttrell (1987) who found that pyrethroid resistance was inherited as an incompletely recessive trait in budworms. The bollworm estimate (0.98) indicates that pyrethroid resistance is inherited as an almost completely recessive trait.

Results and Discussion

Using estimated, 1999 resistance-allele frequencies as a starting point, figures 1 and 2 present the numbers of years it takes Bt resistance-allele frequencies to reach 0.50 for various constant proportions of Bt cotton planted and for different pyrethroid-use regimes. As expected, years to resistance onset in both insects decrease as the proportion of Bt cotton planted in Louisiana increases. As the proportion of Bt cotton planted declines, the number of years it takes Bt resistance to onset depends critically on the assumption taken regarding pyrethroid use. Years to Bt resistance onset dramatically increase under untreated refuge relative to treated refuge, and resistance onset is most rapid in a hypothetical case in which pyrethroid use is allowed on non-Bt refuge but disallowed on Bt.

Higher proportions of Bt- and pyrethroid-susceptible genotypes survive and reproduce when refuge is untreated. Remarkably, susceptibility to pyrethroids in both insects is regenerated in a few years, leading to a toxin-mixture effect. As a result, the proportions of insects homozygous for Bt and pyrethroid susceptibility remain high for a long time, substantially reducing the rate of resistance development. Under treated refuge, bollworm and budworm pyrethroid resistance is complete in only a few years, eliminating potential toxin-mixture effects on Bt resistance. As a result, the proportions of insects homozygous for Bt and pyrethroid resistance increase steadily. Even though pyrethroid susceptibility is regenerated in the hypothetical case, the toxin-mixture effect has no impact on Bt resistance because pyrethroid use is disallowed on Bt cotton. In this case, the proportions of insects heterozygous for Bt resistance increase steadily, substantially increasing the rate of resistance development.

Figures 3 and 4 demonstrate the toxin-mixture effect. Figure 3 shows annual time paths of pyrethroid and Bt resistance-allele frequencies for both insects under a constant, five-percent untreated refuge. Likewise, figure 4 shows annual time paths of resistance-allele frequencies under a constant, five-percent treated refuge. Pyrethroid susceptibility is regenerated in approximately five years under untreated refuge, whereas pyrethroid susceptibility is completely eliminated under treated refuge in three to four years. Untreated refuge and the remarkable regeneration of pyrethroid susceptibility dramatically reduce the rate of Bt resistance development in both insects relative to the treated-refuge case.

Summary

The rates of Bt and pyrethroid resistance development in bollworms and budworms may critically depend on whether pyrethroids, or other conventional insecticides, are used on non-Bt refuge acres. According to theoretical models of resistance and the available data, the use of untreated refuge regenerates susceptibility to pyrethroids in both insects, leading to a toxin-mixture effect that significantly reduces the rate of Bt resistance development relative to the treated-refuge case. As shown in companion economic analyses, this has significant implications for the design of Bt refuge policy (Livingston, 1999; Livingston et al., 2000). In particular, we demonstrate that untreated refuge policies dominate treated refuge policies with respect to attainable producer profits in the long run, annual profit volatility, conventional insecticide usage, and end-period resistance-allele frequencies.

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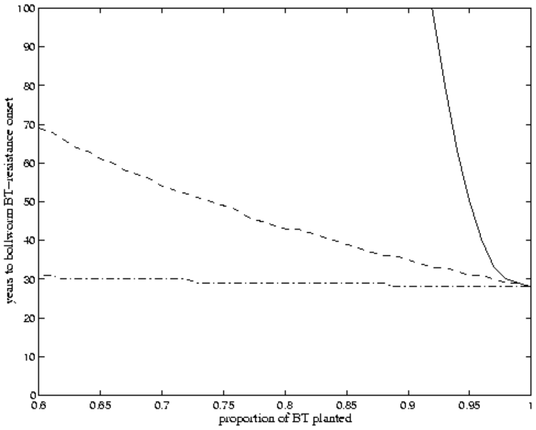
Table 1. Larval genotypes and survival rates facing Bt and pyrethroids on Bt cotton, and pyrethroids used singly on non-Bt cotton.

Genotype	Bt Cotton	Non-Bt Cotton
xy/xy	$(1-\alpha)\ (1-\beta)$	$(1-\alpha)\ (1-\beta)$
xy/xY	$(1-\alpha)\ (1-\gamma\delta_b)$	$(1-\alpha)$
xY/xY	$(1-\alpha)\ (1-\gamma)$	$(1-\alpha)$
xy/Xy	$(1-\beta)\ (1-\epsilon\delta_p)$	$(1-\beta)\ (1-\epsilon\delta_p)$
xy/XY – xY/Xy	$(1-\epsilon\delta_p)\ (1-\gamma\delta_b)$	$(1-\epsilon\delta_p)$
xY/XY	$(1-\epsilon\delta_p)\ (1-\gamma)$	$(1-\epsilon\delta_p)$
Xy/Xy	$(1-\epsilon)\ (1-\beta)$	$(1-\epsilon)\ (1-\beta)$
Xy/XY	$(1-\epsilon)\ (1-\gamma\delta_b)$	$(1-\epsilon)$
XY/XY	$(1-\epsilon)\ (1-\gamma)$	$(1-\epsilon)$

Table 2. Genetic-model, nonlinear least-squares parameter estimates.
Dependent Variable: Average annual pyrethroid survival rate.
Observations: 12.

	Bollworms		Budworms	
Variable	Estimate	P-Value	Estimate	P-Value
α	0.5697	0.6610	0.5107	0.0012
δ_p	0.9834	0.4658	0.8306	0.0104
ϵ	0.9494	0.0000	0.8551	0.0000
x_{1987}	0.0004	0.9973	0.0061	0.8824
R^2	0.7983		0.8824	



igure 1. Years to bollworm Bt-resistance onset for various proportions of Bt cotton planted in Louisiana and various assumptions regarding pyrethroid use.

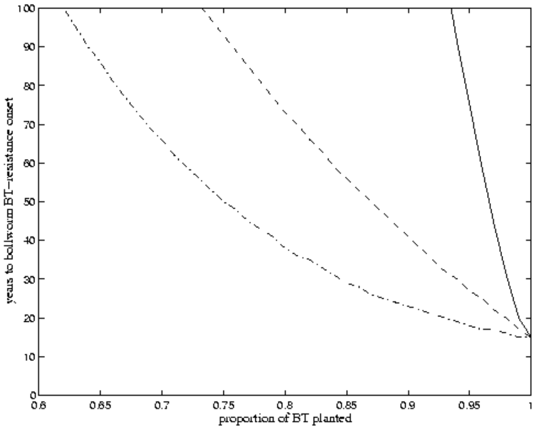


Figure 2. Years to budworm Bt-resistance onset for various proportions of Bt cotton planted in Louisiana and various assumptions regarding pyrethroid use.

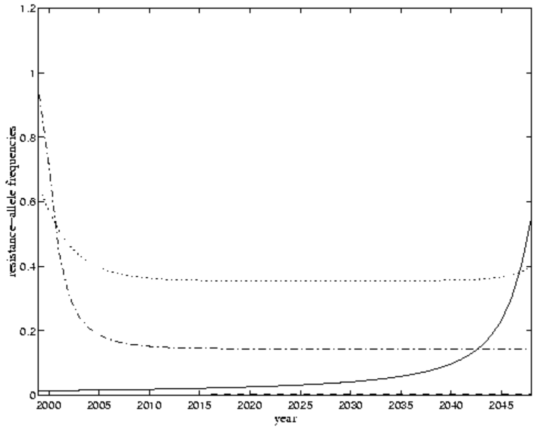


Figure 3. Annual time paths of bollworm Bt (-), budworm Bt (- -), bollworm pyrethroid (•••), and budworm pyrethroid (-•-) resistance-allele frequencies with 95-percent, treated Bt cotton planted and a five-percent, untreated non-Bt refuge: Louisiana.

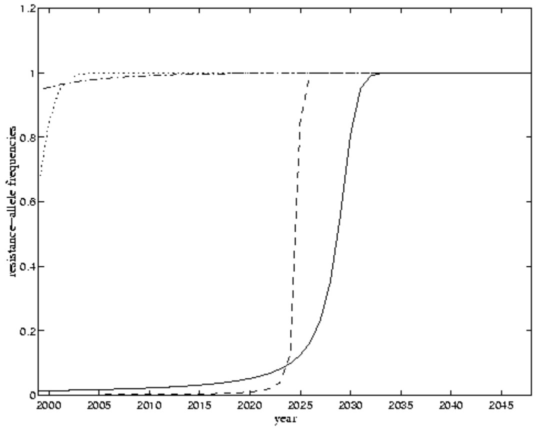


Figure 4. Annual time paths of bollworm Bt (-), budworm Bt (- -), bollworm pyrethroid (•••), and budworm pyrethroid (-•-) resistance-allele frequencies with 95-percent, treated Bt cotton planted and a five-percent, treated non-Bt refuge: Louisiana.