Supplementary Information

Suppressing Resistance to Bt Cotton with Sterile Insect Releases

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Methods: Computer Simulations

As detailed below, we based assumptions primarily on empirical data for pink bollworm. However, to conservatively test the potential for sterile moth releases to delay resistance, we also used some assumptions that overestimate the rate of resistance evolution.

We used a previously described stochastic, spatially explicit model of pink bollworm resistance to Bt cotton[35] with some modifications. We modeled scenarios where resistance would evolve with limited refuges and no sterile releases, as reported for pink bollworm resistance to Bt cotton producing Cry1Ac in India[5] and for some cases with other pests[3]. Supplementary Table 1 summarizes the parameter values that we examined.

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Larval survival was controlled by one locus with two alleles, resistant (*r*) and susceptible (*s*). This is a simplified, but reasonable representation of pink bollworm resistance to Bt cotton that produces Cry1Ac. In several lab-selected strains of pink bollworm from Arizona that survive on Bt cotton producing Cry1Ac, resistance is tightly linked with three mutant alleles at a single cadherin locus[39-41]. The initial *r* allele frequency (0.018) was the mean of estimates from Arizona field populations of pink bollworm based on bioassays from 1997 to 2004 and DNA screening from 2001 to 2005[7, 8, 41]. Genotype-specific larval survival was based on experimental evidence from greenhouse experiments with pink bollworm on cotton plants[7, 8, 11, 13, 15]. Based on this experimental evidence, all simulations incorporated a moderate, recessive fitness cost of resistance (15%) and incomplete resistance (Supplementary Table 1). As observed experimentally for pink bollworm[7, 8, 11, 13, 15], inheritance of resistance was recessive (h = 0) in the simulations summarized in Fig. 1. In simulations of a hypothetical worst-case scenario, resistance was dominant (h = 1) (Supplementary Fig. 1).

We modeled pink bollworm's interaction with Bt cotton producing Cry1Ac. This mimics the field situation in Arizona from 1996 to 2004, when >99% of the state's Bt cotton produced only Cry1Ac. This is also a reasonable, conservative simplification for Arizona from 2005 to 2009, when the area planted to Bt cotton each year consisted of a mean of 57% (range = 21 to 89%) of cultivars producing only Cry1Ac and 43% (range =11 to 79%) of cultivars producing toxins Cry1Ac and Cry2Ab[46-50]. Both types of Bt cotton have essentially 100% efficacy against susceptible pink bollworm larvae[11, 51, 52]. Thus, we can exclude the hypothesis that use of cotton producing two toxins caused the dramatic declines in pink bollworm population density. As explained below, modeling only one-toxin Bt cotton rather than the mosaic of one- and two-toxin Bt cotton that occurred in Arizona from 2005 to 2009, probably overestimates the rate of evolution of resistance.

Experimental evidence shows that Bt cotton producing both Cry1Ac and Cry2Ab can kill pink bollworm larvae resistant to Cry1Ac[42, 51]. Furthermore, theoretical analyses and greenhouse experiments with a model system show that transgenic crops producing two distinct Bt toxins can delay resistance longer than those producing one Bt toxin[53]. Nevertheless, theory and data indicate that mosaics of one- and two-toxin Bt cultivars accelerate evolution of resistance to both toxins relative to planting only a two-toxin Bt cultivar, and thus eliminate much of the advantage of the two-toxin Bt cultivar[53, 54]. Additionally, in greenhouse experiments with a mosaic of one- and two-toxin Bt cultivars in a 1:1 ratio, resistance evolved much faster to the toxin in the one-toxin Bt cultivar than to the toxin present only in the two-toxin Bt cultivar[54]. Therefore, relative to planting only a one-toxin cultivar, mosaics are expected to provide a small delay in evolution of resistance to the toxin in the one-toxin Bt cultivar. In the field, pink bollworm resistance to Bt cotton producing only Cry1Ac evolved rapidly in India where a mosaic of one- and two-toxin Bt cotton was planted[5]. Widespread pink bollworm resistance to Bt cotton producing only Cry1Ac was detected in Gujarat, India in 2009, where more than 65% of growers planted cotton producing both Cry1Ac and Cry2Ab in that year[5].

We modeled a square region of 400 cotton fields; each field was 15 hectares. For each simulation, the percentage of fields planted with non-Bt cotton refuges was 0, 2, 5, 10, 15 or 20%. For each simulation, the location of Bt and non-Bt cotton fields was chosen randomly and remained fixed across years.

The time step in the model was a day, with insect and plant phenology based on accumulation of heat units in degree-days[35]. Based on spermatophore counts in field-collected females, females mated once[55, 56]. Adults moved at most once, with 45% staying in their natal field, 54% moving to an adjacent field, and 1% moving two fields away from their natal field[57]. In addition to density-independent mortality (Supplementary Table 1), we included a density-dependent reduction in fecundity to avoid unrealistically high populations in refuges. We used the following equation, which is modified from equation 3 of Gilpin and Ayala[58]:

$$F = 15 \times \{\text{maximum } (1 - [N/K]^{10}), 0\}$$

where F is the eggs laid per female per day, N is the number of larvae per field and K is the carrying capacity (4,200,000 larvae per field). This density dependence had little effect unless the larval population size exceeded 75% of the carrying capacity (e.g., with N/K = 0.75, F = 15 X 0.94 = 14). Fecundity was zero for a given day if the population size that day was equal to or greater than the carrying capacity.

In simulations with sterile moths, releases occurred in all fields once every 3 days for 27 weeks (May 1 - October 15), yielding a total of 56 releases per year. Mating was random among sterile and wild moths. Sterile moths were the same as wild moths in terms of their survival, dispersal, and sex ratio (1:1). Wild females that mated with sterile males produced no offspring. Various tests have showed that sterile pink bollworm moths and their wild counterparts have similar traits, including mating behavior[59]. Nonetheless, we cannot exclude the possibility that sterile moths had deficits in behavior or longevity that reduced their efficacy. To address this possibility, we simulated a range of sterile moth release rates that were much lower than the actual rates in the field from 2006 to 2009. The range was chosen based on pilot simulations exploring the lowest release rates that had a substantial impact.

The rate of sterile releases was 10 times higher in non-Bt cotton fields than in Bt cotton fields, which is similar to the ratio used in the eradication program (see Sterile Releases, below). In simulations with recessive resistance, the numbers of sterile moths per field per release were: a) 0; b) 1 in Bt cotton and 10 in non-Bt cotton; and c) 4 in Bt cotton and 40 in non-Bt cotton. Given that each simulated field was 15 ha and simulated releases occurred once every 3 days, the simulated release rates in terms of moths per ha per week were a) 0; b) 0.16 in Bt cotton and 1.6 in non-Bt cotton; and c) 0.62 in Bt cotton and 6.2 in non-Bt cotton. In simulations under the worst-case scenario of dominant inheritance of resistance, the simulated release rates in moths per field per release were d) 0; e) 250 in Bt cotton and 2500 in non-Bt cotton; and f) 500 in Bt cotton and 5000 in non-Bt cotton. In terms of sterile moths per ha per week these were d) 0; e) 39 in Bt and 390 in non-Bt; and f) 78 in Bt cotton and 780 in non-Bt cotton. The actual mean release rates from 2006

to 2009 (see Sterile Releases, below) were more than 600 times higher than the highest rate simulated with recessive resistance and about 5 times higher than the highest rate simulated with dominant resistance.

In simulations with refuges, the initial ratio of sterile moths to wild moths was much higher in Bt cotton fields than in non-Bt cotton fields, even though the absolute sterile release rate was 10 times higher in non-Bt cotton fields than in Bt cotton fields. This occurred because initial larval survival and abundance of wild moths was much lower in Bt cotton fields. With the initial resistance allele frequency of 0.018, the initial frequency of resistant homozygotes (*rr)* was 0.000324. With recessive inheritance, survival on Bt cotton was 15% for *rr* larvae and 0% for heterozygous (*rs)* and homozygous susceptible (*ss*) larvae, yielding an initial larval population survival rate on Bt cotton of 0.0000486 (0.000324 X 0.15). In contrast, the initial larval population survival rate on non-Bt cotton was 0.208 (determined primarily by the survival rate of *ss* and *rs* larvae). Therefore, the initial larval population survival rate was 4280 times higher on non-Bt cotton than Bt cotton. Taking into account the 10-fold higher release rate on non-Bt cotton and ignoring movement between Bt and non-Bt cotton fields, the initial ratio of sterile moths to wild moths was about 400 times higher in Bt cotton fields relative to non-Bt cotton fields. Although mating was random within fields, movement between fields was limited, as described above. With the higher ratio of sterile to wild moths in the Bt cotton fields, the probability of mating with a sterile moth was higher for *rr* moths than for *rs* or *ss* moths. Following a similar line of reasoning, under the worst-case scenario of dominant inheritance of resistance, the initial larval population survival was 0.00535 in Bt cotton, and the initial ratio of sterile moths to wild moths was about four times higher in non-Bt cotton than in Bt cotton. With sufficiently high sterile release rates, the population size in Bt cotton fields remained close to 0 and these fields were repeatedly re-colonized by moths from non-Bt cotton fields.

Simulations lasted at most 20 years and stopped sooner if resistance criteria were met or the regional population size was zero. The criteria for resistance were an *r* allele frequency >0.50 and an overwintered population size of >2885 larvae per field, which is 10% of the initial population size (28,850 overwintered larvae per field) and 0.07% of the carrying capacity (4,200,000 larvae per field). We included the population size criterion in addition to the standard *r* allele frequency criterion because in some cases, the *r* allele frequency exceeded 0.50 but the regional population size subsequently declined to zero. This occurred only with recessive inheritance of resistance and sterile releases. In most cases, incorporation of the population size criterion added at most two years to the recorded time for resistance to occur and had little effect on qualitative outcomes.

We used sensitivity analyses to examine the effects of sterile moth releases, percentage of refuges, and the interaction between these two factors. Ten replicate simulations were conducted with each set of parameter values. In most cases, variation was limited among the 10 replicates, but some cases included outcomes where resistance occurred in 20 years or less in some replicates but did not occur in others. To accommodate such cases, we calculated the median number of years until resistance occurred for each set of 10 replicates.

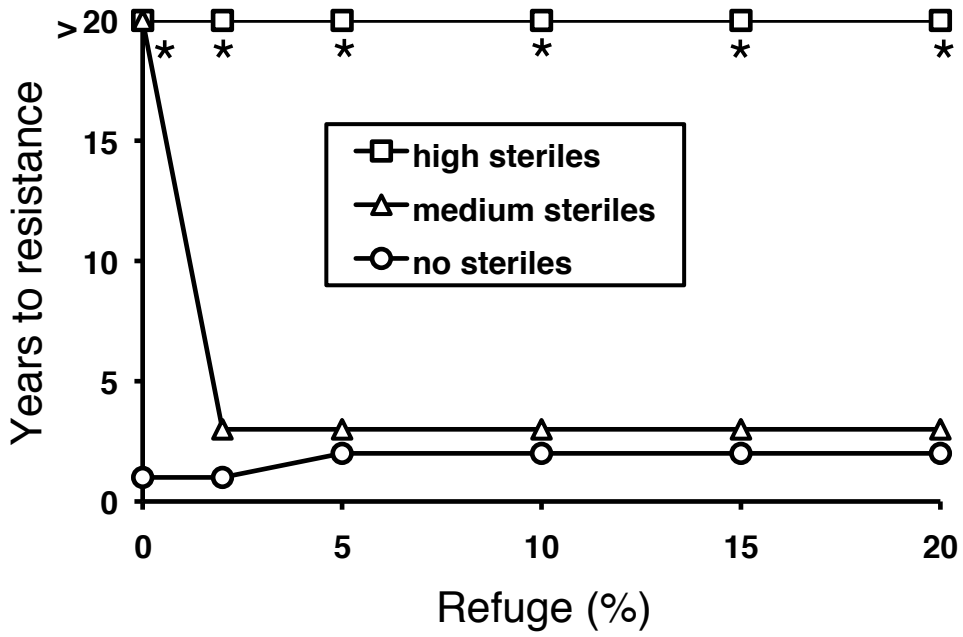
Supplementary References

46. USDA Agricultural Marketing Service - Cotton Program. Cotton Varieties Planted: 2005 Crop. http://search.ams.usda.gov/mndms/2005/08/cn20050831avar.pdf
47. USDA Agricultural Marketing Service - Cotton Program. Cotton Varieties Planted: 2006 Crop. http://search.ams.usda.gov/mndms/2006/08/cn20060831avar.pdf
48. USDA Agricultural Marketing Service - Cotton Program. Cotton Varieties Planted: 2007 Crop. http://search.ams.usda.gov/mndms/2007/08/cn20070830AVAR.pdf
49. USDA Agricultural Marketing Service - Cotton Program. Cotton Varieties Planted: 2008 Crop. http://search.ams.usda.gov/mndms/2008/09/CN20080908AVAR.PDF
50. Agricultural Marketing Service - Cotton Program. Cotton Varieties Planted: 2009 Crop. http://www.ams.usda.gov/mnreports/cnavar.pdf
51. Tabashnik, B. E. *et al.* Control of resistant pink bollworm by transgenic cotton with *Bacillus thuringiensis* toxin Cry2Ab. *Appl. Environ. Microbiol.* **68,** 3790-3794 (2002).
52. Ellsworth, P. C., Moser, H., Henneberry, T., Majeau, G. & Subramani, J. "Transgenic comparisons of pink bollworm efficacy and response to heat stress," (In J. C. Silvertooth [ed.], Cotton, A College of Agriculture and Life Sciences Report. Publ. No. AZ1283. University of Arizona, Tucson, AZ. pp. 147-157, 2002; http://cals.arizona.edu/pubs/crops/az1283/az12835b.pdf).
53. Zhao, J.-Z. *et al.* Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. *Nature Biotechnol.* **21**, 1493-1497 (2003).
54. Zhao, J.-Z. *et al.* Concurrent use of transgenic plants expressing a single and two *Bacillus thuringiensis* genes speeds insect adaptation to pyramided plants. *Proc. Nat'l. Acad. Sci. USA* **102,** 8426-8430 (2005).
55. Ouye, M. T., Graham, H. M., Richmond, C. A. & Martin, D. F. Mating studies of the pink bollworm. *J. Econ. Entomol.* **57,** 222-225 (1964).
56. Graham, H. M. Glick, P. A., Ouye, M.T. & Martin, D. F. *Annals Entomol. Soc. Amer.* **58**, 595 (1965).
57. Sisterson, M. S., Carrière, Y., Dennehy, T. J. & Tabashnik, B. E. Evolution of resistance to transgenic crops: interactions between insect movement and field distribution. *J. Econ. Entomol.* **98,** 1751-1762 (2005).
58. Gilpin, M. E. & Ayala, F. J. Global models of growth and competition. *Proc. Nat'l. Acad. Sci. USA* **70,** 3590-3593 (1973).
59. Miller, T. A., Miller, E., Staten, R. & Middleham, K. Mating response behavior of sterile pink bollworms (Lepidoptera: Gelechiidae) compared with natives. *Environ. Entomol.* **87,** 680-686 (1994).

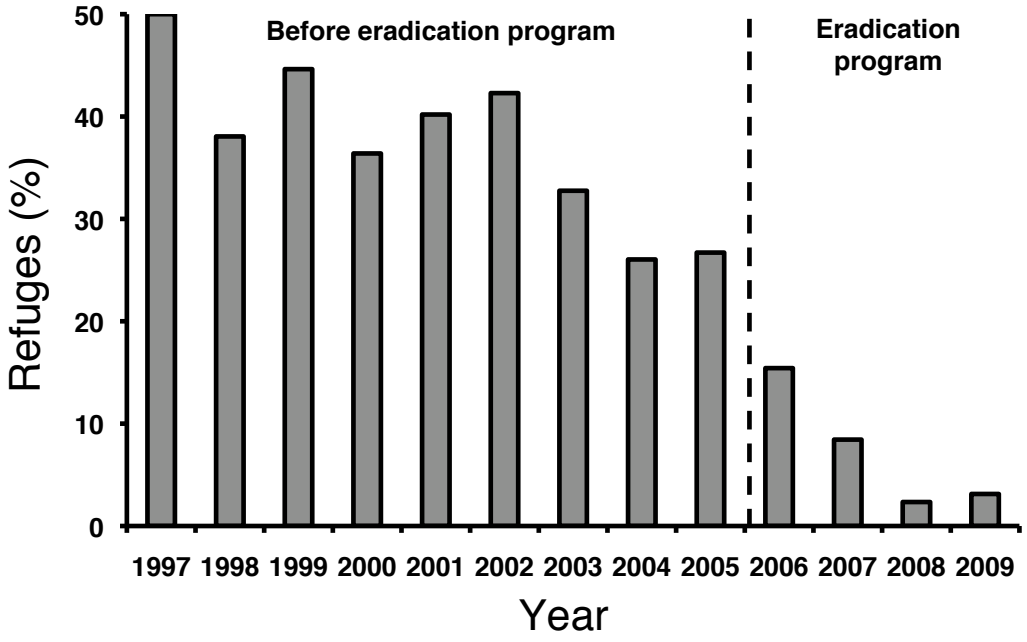
Supplementary Table 1. Parameter values for computer simulations. Parameters identified as means were stochastic, based on binomial distributions[33].

Parameter	Value(s)
Genetics of resistance	
Initial resistance (*r*) allele frequency (mean)	0.018
Larval survival (mean %)	
ss and *rs* on non-Bt cotton	20.8
rr on non-Bt cotton	17.7
ss on Bt cotton	0
rs on Bt cotton: recessive resistance	0
rs on Bt cotton: dominant resistance	15
rr on Bt cotton	15
Cotton fields	
Number of fields	400 (20 X 20)
Size of fields	15 hectares
Fields planted as non-Bt cotton refuges (%)	0, 2, 5, 10, 15, 20
Population dynamics	
Initial population size (mean overwintered larvae per field)	28,850
Carrying capacity (larvae per field)	4,200,000
Sex ratio (mean)	1:1
Adults leaving their natal field (mean %)	55
Daily adult survival (mean %)	85
Maximum eggs per female per day	15
Egg-pupa development time (mean degree-days)	433
Larval overwintering survival (mean %)	5
Sterile moths	
Release period	May 1-Oct 15 (24 weeks)
Frequency of releases for each field	1 per 3 days (56 per year)
Sterile moths per field* per release	
Recessive resistance (Fig. 1)	0; 1 Bt & 10 non-Bt; 4 Bt & 40 non-Bt
Dominant resistance (Supplementary Fig. 1)	0; 250 Bt & 2500 non-Bt; 500 Bt & 5000 non-Bt

*See text for conversion from sterile moths per field per release to sterile moths per ha per week



Supplementary Fig. 1. Evolution of resistance to Bt cotton in computer simulations with a worst-case scenario of dominant inheritance of resistance. Assumptions were the same as in Fig. 1, except for the following changes in larval survival and sterile release rates: Larval survival on Bt cotton was 15% for heterozygotes (*rs*) (same as for *rr*). We simulated three sterile release rates (units are moths per ha per week): 0 (○); medium (△) = 39 in Bt cotton and 390 in non-Bt cotton; and high (□) = 78 in Bt cotton and 780 in non-Bt cotton. Each point represents the median of 10 simulations. Asterisks indicate the regional population size declined to zero. At the high release rate, the regional population size declined to zero in 2 to 5 years in all replicates across the refuge percentages of 0 to 20%. At the medium release rate and no refuges, the regional population size declined to zero in 2 years. With the medium release rate, refuges prevented loss of the regional population and the median time to resistance was 3 years with refuges of 2, 5, 10, 15 and 20%. The sterile release rate (moths per ha per week) at which resistance did not occur in 20 years across all refuge percentages examined (0 to 20%) was 125 times higher with dominant inheritance (78 in Bt cotton and 780 in non-Bt cotton) than with recessive inheritance (0.62 in Bt cotton and 6.2 in non-Bt cotton) (Fig. 1).



Supplementary Fig. 2. The percentage of cotton planted to non-Bt cotton refuges in Arizona from 1997 to 2009. The refuge percentage includes non-Bt cotton planted by farmers who planted no Bt cotton.