



Transgenic cotton and sterile insect releases synergize eradication of pink bollworm a century after it invaded the United States

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Edited by David Denlinger, The Ohio State University, Columbus, OH, and approved November 20, 2020 (received for review September 10, 2020)

Invasive organisms pose a global threat and are exceptionally difficult to eradicate after they become abundant in their new habitats. We report a successful multitactic strategy for combating the pink bollworm (*Pectinophora gossypiella*), one of the world's most invasive pests. A coordinated program in the southwestern United States and northern Mexico included releases of billions of sterile pink bollworm moths from airplanes and planting of cotton engineered to produce insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt). An analysis of computer simulations and 21 y of field data from Arizona demonstrate that the transgenic Bt cotton and sterile insect releases interacted synergistically to reduce the pest's population size. In Arizona, the program started in 2006 and decreased the pest's estimated statewide population size from over 2 billion in 2005 to zero in 2013. Complementary regional efforts eradicated this pest throughout the cotton-growing areas of the continental United States and northern Mexico a century after it had invaded both countries. The removal of this pest saved farmers in the United States \$192 million from 2014 to 2019. It also eliminated the environmental and safety hazards associated with insecticide sprays that had previously targeted the pink bollworm and facilitated an 82% reduction in insecticides used against all cotton pests in Arizona. The economic and social benefits achieved demonstrate the advantages of using agricultural biotechnology in concert with classical pest control tactics.

eradication | invasive species | genetically engineered crop | sterile insect technique | *Pectinophora gossypiella*

Invasive life forms pose a major global threat and are especially difficult to eradicate after they become widespread and abundant in their new habitats (1–4). The pink bollworm (*Pectinophora gossypiella*), one of the world's most invasive insects, is a voracious lepidopteran pest of cotton that was first detected in the United States in 1917 (5–8). For most of the past century, it was particularly destructive in the southwestern United States, including Arizona, where its larvae fed almost exclusively on cotton, consuming the seeds inside bolls and disrupting lint production (6, 8). In 1969, its peak seasonal density at an Arizona study site was 1.8 million larvae per hectare (ha), which translates to over 200 billion larvae in the 126,000 ha of cotton planted statewide that year (9, 10). In 1990, this pest cost Arizona cotton growers \$48 million, including \$32 million damage to cotton despite \$16 million spent for insecticides sprayed to control it (11). In several field trials, mass releases of sterile pink bollworm moths to mate with wild moths reduced progeny production somewhat, yet did not suppress established populations because the sterile moths did not sufficiently outnumber the wild moths (6, 12–14).

Pink bollworm control was revolutionized in 1996 by the introduction of cotton genetically engineered to produce insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt). Bt proteins kill some major insect pests yet are not toxic to most nontarget organisms, including people and many beneficial insects (15–17).

Transgenic Bt cotton helped to reduce the total annual cost of pink bollworm damage and insecticide treatments to \$32 million in the United States (18). Although Bt cotton kills essentially 100% of susceptible pink bollworm larvae (19–21), this pest rapidly evolved resistance to Bt proteins in laboratory selection experiments in Arizona and in Bt cotton fields in India (20–24). To delay the evolution of resistance to Bt cotton, farmers in Arizona planted “refuges” of non-Bt cotton that yielded abundant susceptible moths to mate with the rare resistant moths emerging from Bt cotton (Fig. 1A). The refuge strategy, which has been mandated in the United States and many other countries, but was not adopted widely by farmers in India, helped preserve pink bollworm susceptibility to Bt cotton in Arizona from 1996 to 2005 (24).

As part of a coordinated, multitactic effort to eradicate the pink bollworm from the southwestern United States and northern Mexico, a new strategy largely replacing refuges with mass releases of sterile pink bollworm moths was initiated in Arizona during 2006 (Fig. 1B; 24–27). To enable this novel strategy, the US Environmental Protection Agency granted a special exemption from the refuge requirement, which allowed Arizona cotton growers to

Significance

We report eradication of the pink bollworm, one of the world's most damaging crop pests, from the cotton-growing areas of the continental United States and northern Mexico. A coordinated, multitactic program achieved this success a century after the pest invaded both countries. The program included releases of billions of sterile pink bollworm moths from airplanes and planting of cotton engineered to produce insect-killing proteins from the bacterium *Bacillus thuringiensis*. Analysis of computer simulations and 21 y of field data from Arizona indicate these two tactics interacted synergistically to suppress the pest. By eradicating the pink bollworm, the program ended the damage it caused to cotton and the insecticide sprays used to control it, yielding economic, environmental, and social benefits.

Author contributions: B.E.T., L.R.L., P.C.E., T.J.D., L.A., and R.T.S. designed research; L.R.L., P.C.E., G.C.U., J.A.F., S.E.N., X.L., L.A., and R.T.S. performed research; B.E.T. and Y.C. analyzed data; B.E.T. wrote the paper; B.E.T. conducted modeling; and L.R.L., P.C.E., J.A.F., S.E.N., T.J.D., L.A., R.T.S., and Y.C. contributed to revising the paper.

Competing interest statement: B.E.T. and J.A.F. are coauthors of patents on engineered *Bacillus thuringiensis* (Bt) toxins (US10704059) and potentiating Bt toxins (US20090175974A1), respectively. T.J.D. is retired and was previously employed by Monsanto, Bayer CropScience, and BASF.

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²Retired.

This article contains supporting information online at <https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2019115118/-DCSupplemental>.

Published December 28, 2020.

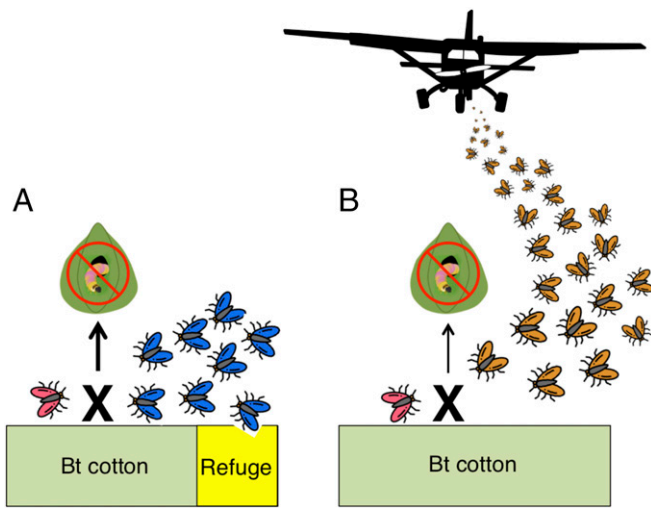


Fig. 1. Management strategies. (A) The refuge strategy is the primary approach adopted worldwide to delay the evolution of pest resistance to Bt crops and was used in Arizona from 1996 to 2005. Refuges of non-Bt cotton planted near Bt cotton produce abundant susceptible moths (blue) to mate with the rare resistant moths (red) emerging from Bt cotton. If the inheritance of resistance to Bt cotton is recessive, as in pink bollworm, the heterozygous offspring from matings between resistant and susceptible moths die when they feed on Bt cotton bolls as larvae (24). (B) Bt cotton and sterile moth releases were used together in Arizona from 2006 to 2014 as part of a multitactic program to eradicate the pink bollworm. Susceptible sterile moths (brown) were released from airplanes to mate with the rare resistant moths emerging from Bt cotton. The few progeny produced by such matings (48) are expected to be heterozygous for resistance and to die when they feed on Bt cotton bolls as larvae.

plant up to 100% of their cotton with Bt cotton (28). We previously reported data from 1998 to 2009 showing that this innovative strategy sustained susceptibility of pink bollworm to Bt cotton while reducing the pest's population density (25). Here, to test the idea of eradicating pink bollworm with the combination of Bt cotton and sterile releases, we conducted computer simulations and analyzed field data collected in Arizona from 1998 to 2018.

Results

Simulated Effects of Bt Cotton and Sterile Insect Releases. In computer simulations of the eradication program in Arizona from 2006 to 2010, pink bollworm was eliminated by the combination of Bt cotton and sterile insect releases, but not by either of the two tactics used alone (Fig. 2). In a realistic scenario, with all model parameters based on empirical data for pink bollworm in Arizona (SI Appendix, Table S1), the population decreased from 200 million to zero in 3 y with Bt cotton and sterile releases deployed together (Fig. 2A). In this scenario, Bt cotton was the primary factor causing the initial declines. These initial decreases dramatically increased the ratio of sterile to wild moths, spurring the population crash in 2008 and 2009 (Fig. 2A). By contrast, under the hypothetical scenario of sterile releases used without Bt cotton, the population grew until reaching its carrying capacity (Fig. 2A). This simulation result is consistent with previous results showing that sterile releases alone did not effectively suppress established field populations of pink bollworm because the sterile moths did not sufficiently outnumber the wild moths (6, 12–14). Under the hypothetical scenario of Bt cotton used without sterile releases, the population size declined by a factor of 0.78-fold each generation (Fig. 2A). Extrapolating this rate indicates 15.6 y would have been required to reduce the population from 200 million to zero, greatly increasing the probability pink bollworm would evolve resistance to Bt cotton before eradication occurred. Assuming that

Bt cotton and sterile insect releases interact additively, the projected time to achieve eradication is also 15.6 y because the sterile insect releases alone did not decrease population size. Thus, the simulated outcome of eradication in 3 y with Bt cotton and sterile releases combined indicates synergy between these two tactics.

In a second set of simulations, we increased the population growth rate per generation (R_o) from 1.6 to 3.2 and the effective number of sterile moths released per generation (S_{eff}) from 3 million to 120 million while keeping all other values the same as in the realistic scenario described above. As in the realistic scenario, the combination of Bt cotton and sterile releases caused eradication in 3 y (Fig. 2B). However, with the higher R_o , the population increased to its carrying capacity with either Bt cotton or sterile releases alone (Fig. 2B). Additional sensitivity analyses imply that under a wide range of reasonable assumptions, the combination of Bt cotton and sterile releases can interact synergistically to rapidly eradicate pink bollworm (SI Appendix, Table S1 and Figs. S1–S5).

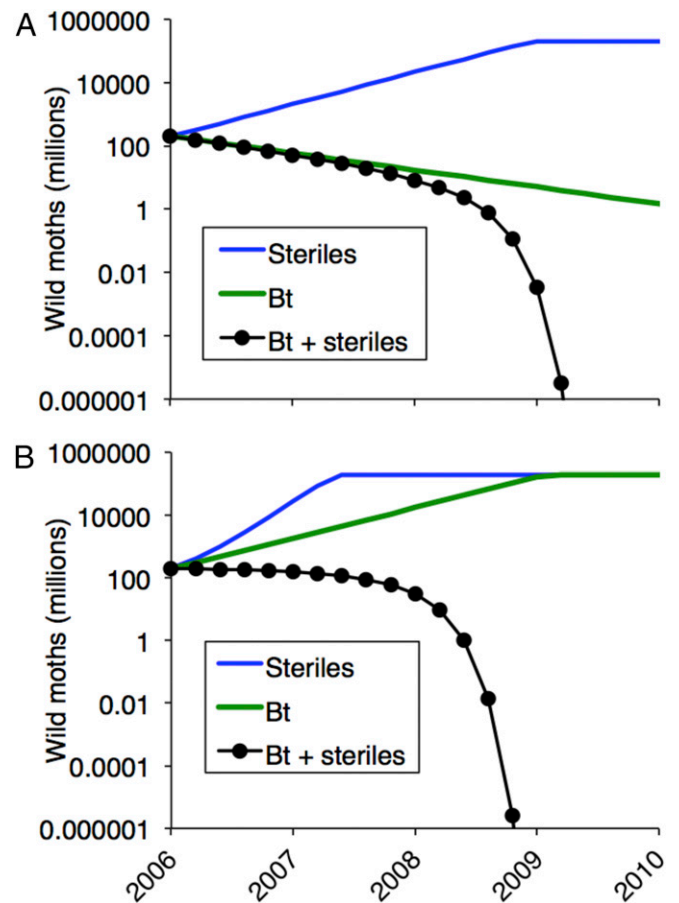


Fig. 2. The simulated effects of sterile moth releases, Bt cotton, and both tactics combined on the population dynamics of pink bollworm. (A) The simulations with realistic values based on empirical data for Arizona for all parameters: initial population size (N_0) = 200 million wild moths, proportion of cotton planted to Bt cotton (p_{Bt}) = 0.93, population growth rate per generation (R_o) = 1.6, proportion of moths emigrating out of the field from which they emerged (e) = 0.55, and the effective number of sterile moths released per generation (S_{eff}) = 3 million (SI Appendix, Table S1). (B) Conditions as in A, except R_o = 3.2 and S_{eff} = 120 million. Eradication is indicated by the lowest value for moths on the y axis (0.000001×1 million = 1 moth). The population stops growing when it reaches the carrying capacity of 200 billion moths.

Observed Effects of the Eradication Program on Pink Bollworm. For 2005, the year before the eradication program began, we calculated that 2.6 billion pink bollworm larvae occurred statewide based on 15.3% of non-Bt cotton bolls infested and 24,754 ha of non-Bt cotton planted in Arizona that year (*SI Appendix, Table S1*). During the eradication program from 2006 to 2014, 11.4 billion sterile pink bollworm moths were released by airplane over cotton fields throughout Arizona, with a yearly mean of 1.6 billion from 2006 to 2012 (range: 1.1 to 2.0 billion, *SI Appendix, Table S2*). The mean annual statewide percentage of cotton planted with non-Bt cotton dropped from 36% before the eradication program (1998 to 2005) to 6% during the eradication program (2006 to 2014; *SI Appendix, Fig. S6*).

Consistent with the simulated effects of Bt cotton and sterile releases combined (Fig. 2), pink bollworm abundance declined to zero during the eradication program (Fig. 3 *A* and *B*), and the population growth was negatively associated with the ratio of sterile to wild males (Fig. 4 and *SI Appendix, Table S3*). The percentage of non-Bt cotton bolls infested with pink bollworm larvae dropped from 15.3% in 2005 to 0.012% in 2009 (two larvae in 16,600 bolls) and then to 0% in 86,413 bolls screened from 2010 to 2018 (Fig. 3*A*). The mean number of wild pink bollworm male moths caught per trap per week fell from 26.7 in 2005 to 0.000012 in 2012 and then to 0 in 188,881 traps checked from 2013 to 2018 (Fig. 3*B*). As expected (29), pink bollworm abundance in bolls and traps were correlated ($r = 0.96$, degrees of freedom (df) = 19, $P < 0.0001$) and detection was more effective with traps than bolls when the population density was extremely low (Fig. 3 *A* and *B*).

The dramatic decrease in abundance during the eradication program contrasts with the period before the eradication program began, when no significant decrease in pink bollworm abundance occurred statewide from 1998 to 2005 (Fig. 3 *A* and *B*). The stability of pink bollworm abundance from 1998 to 2005, when non-Bt cotton accounted for a mean of 36% of all cotton (*SI Appendix, Fig. S6*), is consistent with previous results indicating declines occurred within 15 regions of Arizona only where non-Bt cotton accounted for 35% or less of all cotton (30).

In addition to Bt cotton and sterile insect releases, the eradication program included cultural control tactics for all cotton and the application only in non-Bt cotton fields of pink bollworm female sex pheromone to disrupt mating (18, 25–27 and *SI Appendix*). The cultural control tactics included constraints on planting and harvesting dates to impose a host-free period and postharvest destruction of cotton residues to reduce pink bollworm overwintering survival (*SI Appendix*). Even though the cultural control tactics probably contributed to population suppression, they were similar before and during the eradication program. Thus, it is unlikely they were a primary factor causing the dramatic declines during the eradication program. The pheromone treatments were made only in non-Bt cotton fields, which accounted for a mean of 7% of all cotton ha planted statewide from 2006 to 2011 (*SI Appendix, Fig. S6*). Consistent with the results in Fig. 4, a multiple regression analysis indicated a significant negative association between population growth and sterile insect releases ($P = 0.0002$), whereas the negative association between population growth and pheromone treatments was not significant ($P = 0.49$; *SI Appendix, Tables S4 and S5*).

Economic Impact of Eradication. Concomitant with the dramatic decline in pink bollworm population density, the economic cost associated with the pest plummeted. The mean annual cost of pink bollworm to Arizona cotton growers, including insecticide treatments and yield losses, fell from \$18 million for 1990 to 1995 before Bt cotton was introduced to \$5.4 million for 1996 to 2005 before the eradication program, \$385,000 for 2006 to 2007, and \$0 for 2008 to 2018 (Fig. 3*C*, 11, 25). Sustaining the efficacy of Bt cotton and ending the insecticide sprays targeting pink bollworm facilitated the implementation of integrated pest management

that reduced insecticide treatments for all cotton pests by 82% from 2014 to 2019 relative to 1995 (31, 32). This saved Arizona cotton growers over \$500 million in the past two decades and avoided 11.4 million kilograms of insecticide treatments (31, 32).

Discussion

The results here show that the multitactic eradication program reduced the pink bollworm population in Arizona from more than 2 billion in 2005 to zero in 2013 to 2018. Although we cannot exclude potential contributions to population suppression by cultural control tactics or pheromone treatments, the modeling results (Fig. 2 and

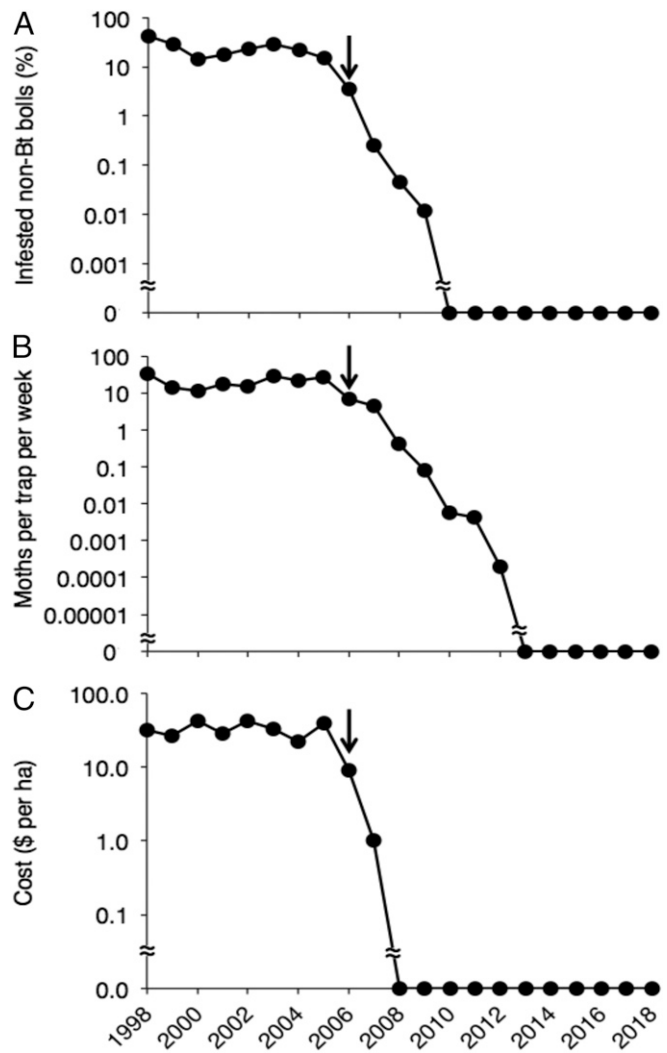


Fig. 3. The eradication program in Arizona reduced the pink bollworm population density and the costs associated with this pest. The arrows indicate the first year of the eradication program (2006). The log scale on the y axis has breaks to allow for the plotting of zero values. (A) Infestation of non-Bt cotton bolls by pink bollworm larvae (log [percent infested non-Bt cotton bolls]) decreased significantly during the eradication program from 2006 to 2009 ($y = -0.70x + 1,407$, $R^2 = 0.99$, $df = 2$, $P = 0.0036$) but not before the eradication program from 1998 to 2005 ($y = -0.031x + 64.1$, $R^2 = 0.23$, $df = 6$, $P = 0.23$). (B) Wild male pink bollworm moths trapped in cotton fields (log [males per trap per week]) decreased significantly from 2006 to 2012 ($y = -0.88x + 1,757$, $R^2 = 0.94$, $df = 5$, $P = 0.0003$) but not from 1998 to 2005 ($y = 0.018x - 34.6$, $R^2 = 0.07$, $df = 6$, $P = 0.52$). (C) The cost of insecticide treatments made against pink bollworm and the yield loss caused by the pest decreased to \$0 from a mean of \$33 per ha of cotton for 1998 to 2005. Boll and trap data from 1998 to 2009 were reported previously (25).

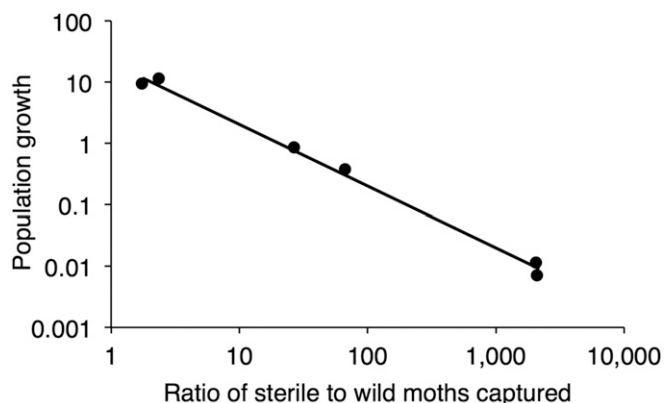


Fig. 4. Pink bollworm population growth decreased as the ratio of sterile to wild moths increased. Linear regression of log-transformed data: $\log [y] = -1.0 \times \log [x] + 1.4$, $R^2 = 0.99$, $df = 4$, $P < 0.0001$. Each point shows results from one year based on male pink bollworm moths captured in Arizona from 2006 to 2011. The ratio on the x axis is from yearly totals (SI Appendix, Table S2). Population growth is the mean number of males trapped per week for the last 5 wk of the season minus the first 5 wk of the season (SI Appendix, Table S5 and Fig. S7).

SI Appendix, Figs. S1–S5) suggest that the synergistic effects of Bt cotton and sterile moth releases were sufficient to eliminate pink bollworm in Arizona within the time frame that eradication actually occurred (Fig. 3). Moreover, the strong negative relationship between population growth and the ratio of sterile to wild males captured (Fig. 4 and SI Appendix, Table S5) supports the idea that variation in this ratio was a key determinant of population dynamics.

We focus here on Arizona because, among the states included in the eradication program, its cotton was most damaged by the pink bollworm and its data collection was most comprehensive. However, parallel efforts in California, New Mexico, and Texas in the United States and Baja California, Chihuahua, and Sonora in Mexico were also essential for regional removal of this pest (18, 27, 33). This program benefited from a strong grower commitment; cooperation among scientists in government, academia, and industry; a well-developed infrastructure for monitoring pink bollworm; virtually 100% efficacy of Bt cotton against the pest; and the pest's almost complete dependence on cotton as a larval host plant (6, 18, 25, 27).

The program's success enabled the declaration by the US Department of Agriculture (USDA) in 2018 that the pink bollworm was eradicated from the cotton-growing regions of the continental United States—a century after this agency published a bulletin about preventing the pest's establishment (34, 35). The cost savings in the United States is \$32 million per year (15), totaling \$192 million from 2014 to 2019. The elimination of pink bollworm from the southwestern United States and northern Mexico contrasts with its continued presence in more than 100 nations worldwide (5). In India, pink bollworm populations soared after evolving resistance to Bt cotton that produces one or two insecticidal Bt proteins (23, 24). Although sustained susceptibility of some pests other than pink bollworm to Bt crops is associated with decreases in their population density (36–38), we are not aware of previous examples where pest eradication has been achieved by a transgenic crop alone or in combination with other tactics. Moreover, in at least 22 documented cases worldwide, nine major pests have evolved practical resistance to Bt crops (24, 39, 40).

Pink bollworm reinfestation of the southwestern United States and northern Mexico is a threat because this pest still occurs

globally in nearly all other regions where cotton is grown, including the Caribbean, South America, and Asia (5). Restrictions on the movement of cotton have been lifted within the United States but remain in effect to thwart the importation of pink bollworm into the United States. Continuing surveillance for pink bollworm includes traps baited with female sex pheromone to detect male moths and screening of non-Bt cotton bolls to detect larvae. Also, cotton growers, crop consultants, and Cooperative Extension personnel are encouraged to immediately report suspected pink bollworm damage or presence. If reinfestation occurs, recommended responses include insecticide and pheromone treatment of all cotton fields within 1.6 km of the infestation and increased trapping within 23 km² to determine the extent of the infestation. Reinfestation by pink bollworm resistant to the Cry1 and Cry2 toxins produced by currently planted Bt cotton would be especially problematic. To counter this threat, small colonies of pink bollworm are being maintained in Arizona under strict quarantine to provide sources from which mass rearing for sterile releases could be reactivated and to continue research to find novel Bt proteins that kill pink bollworm resistant to Cry1 and Cry2 toxins (41).

One potential opportunity for extrapolating the multitactic approach described here is in the Yangtze River Valley of China, where millions of smallholder farmers have planted Bt cotton producing one toxin (Cry1Ac) since 2000 (42). By planting second-generation hybrid cotton that includes 25% non-Bt cotton, farmers there have maintained pink bollworm's susceptibility to Bt cotton while gradually reducing the pest's population density (42). Together with sterile moth releases, a shift there to multitoxin Bt cotton as planted in the United States and elsewhere could accelerate pest suppression while delaying the evolution of resistance (24, 43). The successful combination of Bt cotton and sterile insect releases reported here may also spur other synergistic uses of biotechnology, such as releasing transgenic insects or their symbionts to complement transgenic crops (1, 44, 45).

Materials and Methods

To assess the potential effects on pink bollworm population dynamics of Bt cotton and sterile moth releases used separately or together, we used a deterministic population dynamics model that combined concepts and components from previous models that address each of the two tactics separately (30, 46, 47). We chose this approach for several reasons: to readily test the hypothesis that the two tactics can interact synergistically, to enable the incorporation of realistic biological parameters for pink bollworm in Arizona, to examine the projected outcomes of different assumptions about each of the model's parameters using the same basic model, and to make the modeling results readily verifiable by readers. SI Appendix, Table S1 lists the parameter values we used in simulations, including the standard values and additional values tested in sensitivity analyses where we varied one parameter at a time while holding all other parameters constant. Details about the simulations, eradication program methods, data collection, and statistical analyses are provided in the SI Appendix.

Data Availability. Data are available in the tables of the SI Appendix.

ACKNOWLEDGMENTS. We thank B. Degain, C. Ellers-Kirk, V. Harpold, A. Yelich, and the Arizona Cotton Research and Protection Council staff members for their contributions to the eradication program and R.L. Smith for his thoughtful comments. The mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer. This work was supported by the USDA National Institute of Food and Agriculture 2017-70006-27145, 2018-70006-28881, 2018-67013-27821, and 2020-67013-31924; Cotton Inc.; and the Agricultural Biotechnology Stewardship Technical Committee, a consortium of companies that produce transgenic crop seeds.

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