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Suppressing resistance to *Bt* cotton with sterile insect releases

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Genetically engineered crops that produce insecticidal toxins from *Bacillus thuringiensis* (*Bt*) are grown widely for pest control[1]. However, insect adaptation can reduce the toxins' efficacy[2–5]. The predominant strategy for delaying pest resistance to *Bt* crops requires refuges of non-*Bt* host plants to provide susceptible insects to mate with resistant insects[2–7]. Variable farmer compliance is one of the limitations of this approach. Here we report the benefits of an alternative strategy where sterile insects are released to mate with resistant insects and refuges are scarce or absent. Computer simulations show that this approach works in principle against pests with recessive or dominant inheritance of resistance. During a large-scale, four-year field deployment of this strategy in Arizona, resistance of pink bollworm (*Pectinophora gossypiella*) to *Bt* cotton did not increase. A multitactic eradication program that included the release of sterile moths reduced pink bollworm abundance by >99%, while eliminating insecticide sprays against this key invasive pest.

Transgenic cotton and corn that produce proteins from *Bacillus thuringiensis* (*Bt*) for insect control have been planted on a cumulative total of >200 million ha worldwide since their commercial introduction in 1996 (ref. 1). Although *Bt* crops remain effective against most pest populations, several pests have evolved resistance[2–5]. The main strategy for delaying pest resistance to *Bt* crops promotes survival of susceptible insects by providing host plants that do not produce *Bt* toxins[6,7]. These are commonly referred to as 'refuges'. Ideally, most of the rare, resistant insects emerging from *Bt* crops will mate with the relatively abundant susceptible insects from nearby refuges. If resistance is inherited as a recessive trait, the *Bt* crops will kill the hybrid progeny produced by such matings and evolution of resistance will be substantially slowed[6,7].

Retrospective evaluations of global resistance monitoring data suggest that refuges have delayed pest resistance to *Bt* crops[3,7]. In particular, theoretical and empirical analyses imply that refuges have delayed resistance in pink bollworm (*Pectinophora gossypiella*), one of the world's most destructive pests of cotton[7–10]. This invasive insect, which was first detected in the United States in 1917, feeds almost exclusively on cotton in some parts of the southwestern United States, including Arizona[9,10]. Field and greenhouse data show that transgenic cotton that produces *Bt* toxin Cry1Ac (*Bt* cotton) kills essentially 100% of susceptible pink bollworm larvae[11–14]. However, laboratory selection with Cry1Ac quickly produced several resistant strains of pink bollworm from Arizona that could survive on *Bt* cotton plants[11,12,15]. Furthermore, pink bollworm resistance to *Bt* cotton has been reported in the field in India, where farmer compliance with the refuge strategy has been low[5,16]. In contrast, compliance with the refuge strategy is considered a primary reason why pink bollworm susceptibility to *Bt* cotton did not decrease in the field in Arizona from 1997 to 2005 (refs. 8,17).

As part of a coordinated, multitactic effort to eradicate pink bollworm from the southwestern United States and northern Mexico, a new strategy that replaced refuges with season-long releases of sterile pink bollworm moths was initiated in Arizona in 2006 (refs. 18,19) (Online Methods). Under this new strategy, Arizona cotton growers were permitted to plant up to 100% transgenic cotton that produces either one *Bt* toxin (Cry1Ac) or two *Bt* toxins (Cry1Ac and Cry2Ab)[18,19]. The concept underlying this alternative approach is that if enough sterile moths are released, resistant moths will mate mainly with sterile moths, rather than with fertile, wild moths that are either resistant or susceptible to *Bt*. In principle, this approach has several advantages over the refuge strategy. First, farmers could greatly reduce or eliminate planting of refuges and thus avoid associated complications and yield losses. Moreover, because matings with sterile insects do not produce fertile progeny, this approach could delay resistance that is based on either recessive or dominant inheritance. Unlike the refuge strategy, this approach does not require maintenance of pest populations. It is thus compatible with eradication efforts. To test the idea of delaying resistance with sterile insect releases, we conducted computer simulations and analyzed more than a decade of field data from before and after deployment of this strategy statewide in Arizona.

In the computer simulations, sterile moth releases suppressed resistance to *Bt* cotton by decreasing the pest's population size and

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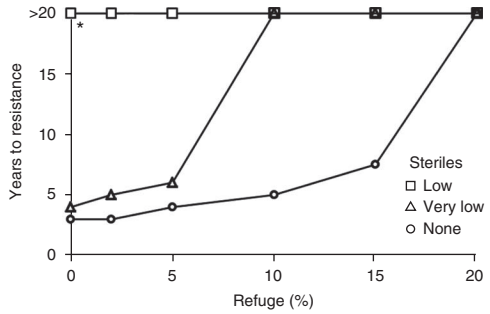

Figure 1 Computer simulations of effects of sterile moth releases on evolution of resistance to *Bt* cotton. We used a stochastic, spatially explicit model with a region of 400 cotton fields of 15 ha each (**Supplementary Methods**). Two alleles ('*r*', resistant; '*s*', susceptible) at a single locus controlled larval survival, which was 0% for *ss* and *rs* (recessive inheritance), and 15% for *rr* on *Bt* cotton; 20.8% for *ss* and *rs*, and 17.7% for *rr* on non-*Bt* cotton. The initial *r* allele frequency was 0.018. The criteria for resistance were an *r* allele frequency >0.50 and a population size >10% of the initial population size. Each point represents the median of ten simulations. The simulated sterile moth release rate was 10 times higher in non-*Bt* cotton fields than in *Bt* cotton fields to mimic field releases. We simulated three sterile release rates: none, very low and low. Sterile release rates (in moths per ha per week) were 0.16 in *Bt* cotton and 1.6 in non-*Bt* cotton for the very low release rate, and 0.62 in *Bt* cotton and 6.2 in non-*Bt* cotton for the low release rate. The actual release rates in the field were >600 times higher than the low release rate (see text). With no refuges and the low release rate, the regional population size decreased to 0 after 2 to 4 years (indicated by asterisk). In the cases with refuges where resistance did not evolve in 20 years, the regional population persisted but the *r* allele frequency remained <0.025 after 20 years.

reducing the probability of mating between resistant moths (**Fig. 1**, **Supplementary Fig. 1** and **Supplementary Methods**). In the simulations, when sufficient numbers of sterile moths were released, pest populations did not persist and resistance did not occur over a 20-year period (**Fig. 1** and **Supplementary Fig. 1**). Based on experimental evidence of pink bollworm responses to *Bt* cotton[11–13,15,17], we first modeled recessive inheritance of resistance with a fitness cost and incomplete resistance (**Fig. 1** and **Supplementary Table 1**). With no refuges, resistance evolved in 3 years without releases of sterile moths, but populations did not persist and resistance did not occur with weekly 'low' releases of 0.62 sterile moths per ha of *Bt* cotton (**Fig. 1**). With refuges accounting for 2 to 20% of the total area planted to cotton, resistance evolved more slowly with increases in the number of sterile moths released, increases in the refuge percentage, or both (**Fig. 1**). With 20% of cotton planted to non-Bt cotton refuges, resistance did not occur in 20 years, even without sterile releases (**Fig. 1**). Because of fitness costs associated with pink bollworm resistance to *Bt* cotton, higher refuge percentages not only reduced the proportion of the population exposed to selection for resistance but also increased selection against resistance[8,20]. In a hypothetical worst-case scenario with dominant inheritance of resistance and no refuges, resistance evolved in 1 year with no sterile moths, but populations did not persist and resistance did not occur with weekly releases of 78 sterile moths per ha of *Bt* cotton (**Supplementary Fig. 1**).

The mean release rate of sterile pink bollworm moths achieved from 2006 to 2009 in Arizona was >600 times higher than the simulated rate that suppressed recessive resistance to *Bt* cotton for >20 years without refuges (**Fig. 1**). For each year from 2006 to 2009, the mean number of sterile moths released per ha per week was 380 (range = 170–830) for *Bt* cotton and 4,400 (range = 3,900–5,200) for non-*Bt* cotton, with a statewide total of 1.7 to 2.1 billion sterile moths released per year. Before sterile releases began in Arizona in 2006, the percentage of cotton planted to non-*Bt* cotton refuges statewide was >25% in all years, with a mean of 37.4% for 1997 to 2005 (**Supplementary Fig. 2**). With the onset of sterile releases, the statewide refuge percentage declined to 15.4% in 2006, 8.4% in 2007, 2.3% in 2008 and 3.1% in 2009 (**Supplementary Fig. 2**).

Consistent with the simulation results (**Fig. 1**), monitoring of pink bollworm field populations showed no net decrease in susceptibility to Cry1Ac from 1997 to 2005, when the refuge percentage was >25% every year, or from 2006 to 2009, when sterile insects were released and the mean refuge percentage was 7.3% (**Fig. 2** and **Supplementary Fig. 2**). DNA screening for the three mutations in the cadherin gene that are linked with pink bollworm resistance to Cry1Ac did not identify any resistant alleles during 2006 to 2009 (n = 2,499) (Online Methods). Based on larval survival on diet treated with Cry1Ac, bioassays detected a single resistant individual during 2006 (n = 3,822), but no resistant individuals were found during 2007 or 2008 (n = 3,602) (**Fig. 2**) (Online Methods). Bioassays also detected no larvae resistant to Cry2Ab during 2007 or 2008 (n = 2,572). As detailed below, in 2009, this pest was so scarce in Arizona that we could not collect enough individuals to conduct bioassays.

Since the eradication program began in 2006, pink bollworm populations have declined dramatically (**Fig. 3**). In 2009, only two pink bollworm larvae were found in 16,600 bolls of non-*Bt* cotton screened statewide. This yields an infestation rate of 0.012%, which represents a 99.9% decline from the 15.3% infestation rate in 2005 (**Fig. 3A**). Likewise, the number of wild male pink bollworm moths caught per trap per week dropped from 26.7 in 2005 to 0.0054 in 2009, a 99.98% decrease (**Fig. 3b**). The decrease in pink bollworm populations during the eradication program was steeper than the decline observed with the planting of *Bt* cotton before the eradication program began in Arizona (**Fig. 3**)[21] and the declines in other target pests associated with planting of *Bt* crops in other regions[22–26].

Along with declines in pink bollworm populations, insecticide sprays against this pest fell to historic lows (**Fig. 4**). The mean number of sprays per ha per year targeting pink bollworm in Arizona was 2.7 from 1990 to 1995, which dropped to 0.64 from 1996 to 2005 with use of *Bt* cotton, before the eradication program (**Fig. 4**)[10,27]. Under the eradication program, this mean decreased to 0.14 in 2006, 0.013 in 2007, 0.0029 in 2008 and 0 in 2009 (**Fig. 4**). The mean yearly cost of pink bollworm to Arizona cotton growers, including

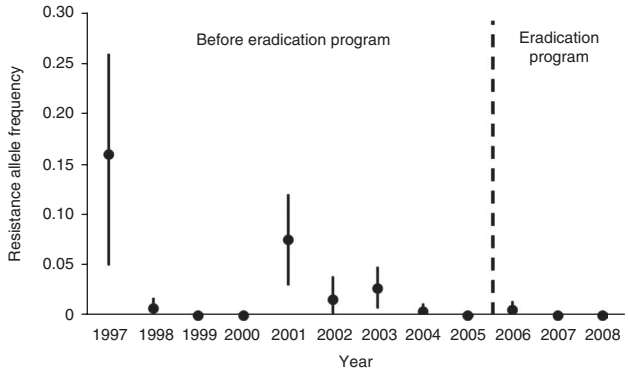

Figure 2 Pink bollworm resistance allele frequency (with 95% confidence intervals) in Arizona from 1997 to 2008, as estimated from laboratory bioassays with Cry1Ac (Online Methods). Data from 1997 to 2004 were reported previously[8].

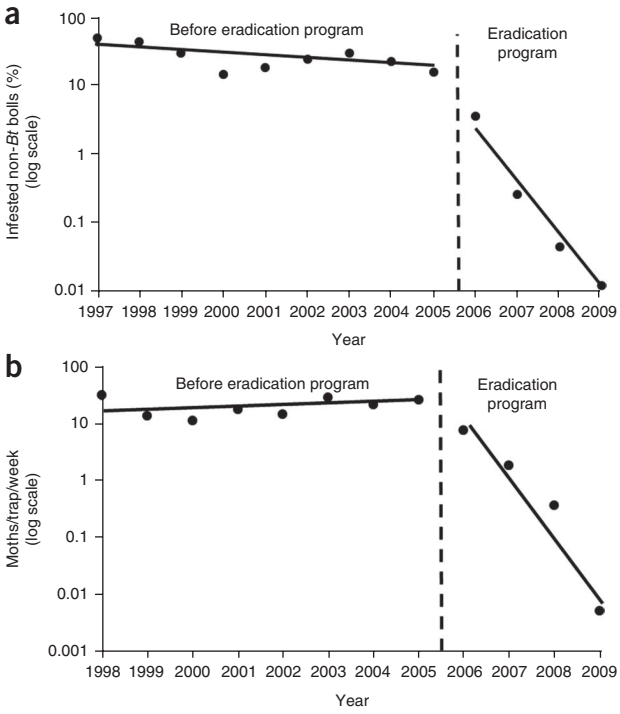

Figure 3 Pink bollworm abundance in Arizona before and during the eradication program. (**a**) Larval infestation of non-*Bt* cotton bolls from 1997 to 2009. Analysis of covariance (Online Methods) shows that infestation (log [% infested non-*Bt* cotton bolls]) was significantly affected by year, treatment (before versus during the eradication program), and a year-by-treatment interaction ($P \leq 0.0001$ for each factor and their interaction, $r^2 = 0.97$). Linear regression shows that the slope, which indicates the decrease in infestation per year, was 18 times steeper from 2006 to 2009 (–0.81, $r^2 = 0.97$, $P = 0.012$) than from 1997 to 2005 (–0.044, $r^2 = 0.42$, $P = 0.059$). Data from 1997 to 2005 were reported previously[14]. (**b**) Wild male pink bollworm moths trapped in *Bt* cotton fields from 1998 to 2009. Analysis of covariance shows that number of moths caught per trap per week (log transformed) was significantly affected by year, treatment (before versus during the eradication program), and a year-by-treatment interaction ($P < 0.0001$ for each factor and their interaction, $r^2 = 0.95$). Linear regression shows that the slope, which indicates the change in moths trapped per year, was significantly negative from 2006 to 2009 (–1.0, $r^2 = 0.92$, $P = 0.04$), but did not differ significantly from 0 from 1998 to 2005 (0.017, $r^2 = 0.071$, $P = 0.52$).

yield losses and insecticide sprays, was $18 million for 1990 to 1995, $5.4 million from 1996 to 2005 and only $172,000 for 2006 to 2009 (ref. 28). By using *Bt* cotton as one component of a comprehensive integrated pest management program, Arizona growers also greatly reduced insecticide use against all cotton pests, including those not killed by *Bt* cotton, saving a cumulative total of $200 million from 1996 to 2009 (refs. 10,27).

The estimated yearly cost of pink bollworm to US cotton growers before the eradication program was $32 million per year[19], which is $2 million more than the mean annual cost of the eradication program in the United States and northern Mexico from 2006 to 2009 (ref. 29). These estimates do not include the value of the indirect advantages of reduced insecticide use associated with the eradication program, such as conservation of natural enemies that control pests other than pink bollworm, and benefits to the environment and human health[27]. Although increasing economic gains are expected if pink bollworm remains scarce and program costs decline, eradication of pink bollworm from the United States and northern Mexico remains challenging because this invasive pest is widespread and resilient[9,10]. Nevertheless, our results show that pink bollworm resistance to *Bt* cotton in Arizona did not increase from 2006 to 2009, despite the low abundance of non-*Bt* cotton refuges. Although simulation results suggest that sterile releases alone can delay resistance to *Bt* crops (**Fig. 1** and **Supplementary Fig. 1**), the dramatic decline in Arizona's pink bollworm population probably reflects the combined effects of the sterile releases, high adoption and sustained efficacy of transgenic cotton producing either one or two *Bt* toxins and other control tactics used in the eradication program[18,19].

Although the sterile insect technique has been known for decades and used successfully in some cases[30,31], the program described here is, to our knowledge, the first large-scale effort using this approach to suppress pest resistance to a transgenic crop. This program has benefitted from a strong grower commitment, public investment in sterile insect technology, a well-developed infrastructure for monitoring pink bollworm resistance and population density, virtually 100% efficacy of *Bt* cotton against pink bollworm, and this pest's nearly exclusive dependence on cotton in Arizona[9,10,18,19]. We do not know whether the success of this program can be replicated with other pests or even with pink bollworm in other parts of the world. Analyses of mathematical models imply that refinements of the sterile insect technique, such as release of transgenic insects carrying a dominant lethal gene, could be more widely applicable for suppression of pests that harm crops or transmit pathogens[31–33]. Our results suggest that further exploration of such tactics could help to enhance the sustainability of *Bt* crops. The results reported here also illustrate the idea that *Bt* crops are likely to be most useful when combined with other tactics for integrated control of pests.

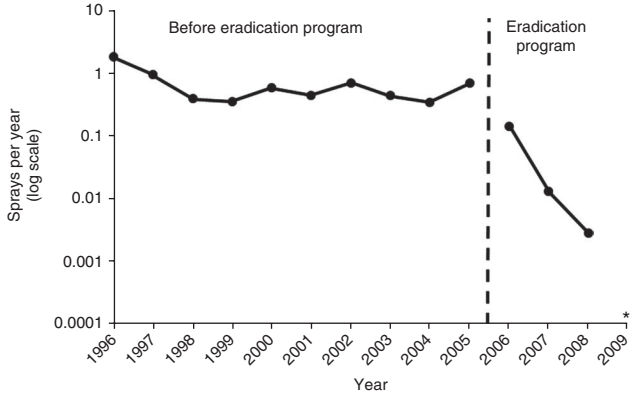

Figure 4 Mean number of insecticide sprays per ha per year targeting pink bollworm on cotton in Arizona from 1996 to 2009. The asterisk indicates 0 sprays in 2009. Analysis of covariance (Online Methods) shows that insecticide use (log [sprays + 0.0001]) was significantly affected by year, treatment (before versus during the eradication program), and a year-by-treatment interaction ($P < 0.0001$ for each factor and their interaction, $r^2 = 0.96$). Linear regression shows that the slope, which indicates the change in sprays per year, was significantly negative from 2006 to 2009 (–1.0, $r^2 = 0.98$, $P = 0.01$), but did not differ significantly from 0 from 1996 to 2005 (–0.035, $r^2 = 0.17$, $P = 0.17$). The regression lines are not plotted because the 0 sprays for 2009 cannot be represented on the log scale used here and the regressions were calculated on a different scale (log [sprays + 0.0001]). Data from 1996 to 2005 were reported previously[27].

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturebiotechnology/.

Note: Supplementary information is available on the Nature Biotechnology website.

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AUTHOR CONTRIBUTIONS

M.S.S. conducted computer simulations; P.C.E. collected and summarized insecticide use data; L.A., L.L., M.W. and R.T.S. directed the eradication program and contributed to its design; J.A.F., G.C.U., A.J.Y., C.E.-K., and V.S.H. collected data; B.E.T., M.S.S., P.C.E., T. J. D., L.A., R.T.S., J.A.F., G.C.U., X. L. and Y.C. contributed to research design; Y.C. analyzed data. B.E.T. wrote the paper. All authors discussed the results and commented on the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

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ONLINE METHODS

Pink bollworm eradication program. The goal of this ongoing program is to eradicate pink bollworm from the United States and northern Mexico[18,19]. The program includes: (i) mapping cotton field locations, sizes and types (*Bt* or non-*Bt*); (ii) measuring pink bollworm abundance by checking cotton bolls for larval infestation and by trapping adult males; (iii) monitoring pink bollworm resistance to *Bt* toxins Cry1Ac and Cry2Ab; and (iv) controlling pink bollworm using *Bt* cotton, sterile moth releases, cultural practices, mating disruption with pheromone in non-*Bt* cotton and minimal insecticide applications. The grower-sponsored Arizona Cotton Research and Protection Council (ACRPC) proposed a plan to allow Arizona cotton growers to plant up to 100% *Bt* cotton that produces either one or two *Bt* toxins, with sterile insect releases instead of non-*Bt* cotton refuges for delaying resistance[18]. The US Environmental Protection Agency (EPA) convened a scientific advisory panel to review the plan[34] and the EPA subsequently approved the plan.

The eradication program began in Texas, New Mexico and Mexico in 2001 and has expanded each year since[19]. It was initiated in phases in Arizona, starting in 2006 with eastern and central Arizona (Cochise, Graham, Greenlee, Maricopa, Pima and Pinal Counties), which accounted for 83% of Arizona's cotton acreage that year. The program extended to northwestern Arizona (La Paz and Mohave Counties) and southern California in 2007, and to southwestern Arizona (Yuma County) and Baja California in 2008. Funding for the binational program is provided by growers (80%) and the USDA-Animal Plant Health Inspection Service (APHIS) (20%)[29].

Computer simulations. To assess the potential effects of sterile moth releases on evolution of resistance to *Bt* cotton, 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]. As detailed in the **Supplementary Methods**, 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. **Supplementary Table 1** summarizes the parameter values that we examined.

Sterile moth releases. The sterile moths released were from the APHIS strain of pink bollworm[36] maintained at the USDA-APHIS Pink Bollworm Rearing Facility in Phoenix, Arizona. This strain originated from Arizona and was infused yearly with wild insects until 2000. Moths were marked internally by rearing larvae on artificial diet containing a fat soluble dye[37] (oil red dye no. 2114, Passaic Color and Chemical Co.). Moths were irradiated with 200 Gy in a Shepherd 484R Cobalt-60 irradiator and stored in containers in groups of 2 million at 4 °C for 1–2 d until release. Moths were released from small airplanes such as Cessna model 206. Each plane had a tube underneath for releasing moths and a device that controlled the release rate. Moths were usually released from dawn to 11 a.m. at an altitude of roughly 150 m and a speed of ~180 km per hour. Throughout each cotton-growing season from 2006 to 2009, 1.7–2.1 billion sterile pink bollworm moths were released over Arizona cotton fields. From May to October, each cotton field received releases two or three times per week. For each year from 2006 to 2009, the mean number of sterile moths released per ha per week was 380 for *Bt* cotton (range = 170–830) and 4,400 (range = 3900–5200) for non-*Bt* cotton. The release rate was higher for non-*Bt* cotton than for *Bt* cotton because larval survival and emergence of wild moths was expected to be higher in non-*Bt* cotton.

Whereas spatial separation between refuges and *Bt* crops could limit the efficacy of the refuge strategy, sterile insect releases were made directly into *Bt* and non-*Bt* cotton fields. Also, although temporal asynchrony in moth emergence between refuges and *Bt* cotton fields could reduce the effectiveness of the refuge strategy[15], sterile releases were made frequently throughout the season, so that sterile moths were available consistently for mating with wild moths.

Refuge percentage. For 1998 to 2009, we determined the total area of cotton (*Gossypium hirsutum* (upland cotton) and *G. barbadense* (Pima cotton)) planted and the area planted to non-*Bt* cotton in Arizona using methods similar to those described previously[17]. We calculated the refuge percentage as the area of non-*Bt* cotton divided by the total area of cotton, multiplied by 100%. Thus, our estimate of the refuge percentage includes non-*Bt* cotton planted by growers who planted no *Bt* cotton. In each year, an experienced field crew trained by the ACRPC mapped the position of cotton fields and collected information from producers on cotton type (*Bt* or non-*Bt*) throughout Arizona. In each year, data collected by field crews were mapped with Geographic Information System (GIS) software and validated by comparing cotton field locations between field-generated paper maps and computer-generated maps. Enzyme-linked immunosorbent assays for Cry1Ac from a randomly chosen subset of fields showed that all of the fields tested had been correctly identified on the GIS map[17]. We analyzed the GIS maps using ArcView software to calculate the area planted to each type of cotton in each year. The statewide *Bt* cotton percentage for 1997 was reported previously[38].

For 1997 to 2009, Arizona's yearly mean total area planted to cotton was 98,000 ha (range = 58,000–123,000 ha). In addition to cotton, pink bollworm larvae in Arizona feed on okra (*Abelmoschus esculentus*), which typically grew on less than 150 ha per year in Arizona, approximately 1/500th (0.2%) or less of the area planted to cotton. We did not include okra in our calculations of non-*Bt* cotton refuges, but sterile moths were released on okra at the same rate used on non-*Bt* cotton.

Resistance monitoring. We monitored pink bollworm resistance to *Bt* toxins Cry1Ac and Cry2Ab using bioassays[8,11]. We also used DNA screening to monitor resistance to Cry1Ac[8,12,13,39–41]. The bioassays can detect resistance caused by any mechanism. Because pink bollworm resistance to the diagnostic concentrations of Cry1Ac and Cry2Ab used in bioassays is recessive[11–13,15,17,42], however, the bioassays do not distinguish between homozygous susceptible larvae and heterozygous larvae carrying only one copy of a resistance gene. The DNA screening detects any of the three mutations in a cadherin gene that are tightly linked with resistance to Cry1Ac in several laboratory-selected strains of pink bollworm from Arizona that survive on *Bt* cotton plants[12,13,39–41]. Although the DNA screening can identify single resistance alleles in heterozygous insects, it detects only the three known cadherin resistance alleles.

Bioassays. To monitor pink bollworm resistance to Cry1Ac and Cry2Ab, we used previously described methods for field sampling, laboratory bioassays and data analysis[8,11,42]. To monitor resistance to Cry1Ac, each year from 1997 to 2008, we tested an average of 2,730 larvae from an average of 11.6 cotton fields in Arizona. The progeny of field-collected pink bollworm from each site were reared and tested separately. Neonates were tested individually for 21 d on artificial diet without toxin (control) or on diet with 10 μg Cry1Ac per ml diet, which kills susceptible homozygotes and heterozygotes but not resistant homozygotes[11]. Based on recessive inheritance of resistance, the Cry1Ac resistance allele frequency for each site was estimated as the square root of the frequency of survivors after adjustment for control mortality[11]. We calculated the 95% confidence interval for each yearly statewide mean resistance allele frequency using the bootstrap method with 10,000 repetitions[8]. Bioassay data from 1997 to 2004 were reported previously[8]. To monitor resistance to Cry2Ab, we used methods similar to those described above for Cry1Ac. Neonates were tested individually for 21 d on artificial diet without toxin (control) or on diet with a diagnostic concentration of 10 μg Cry2Ab per ml diet[42]. The numbers of larvae tested at the diagnostic concentration of Cry2Ab were 2,052 larvae from nine cotton fields in 2007 and 520 larvae from two cotton fields in 2008. The sample size was smaller in 2008 because the scarcity of pink bollworm in that year made it difficult to collect enough live individuals to conduct bioassays.

DNA screening. We used previously described field sampling procedures and allele-specific PCR methods to screen for three cadherin mutations linked with pink bollworm resistance to Cry1Ac[8,12,13,39–41]. Details of the methods and results of DNA screening from 2001 to 2005 were reported previously[41]. DNA screening was completed here for the following numbers of wild pink bollworm individuals collected from the field from Arizona: 1,033 in 2006, 884 in 2007, 364 in 2008 and 218 in 2009 (total = 2,499).

Pink bollworm abundance. Pink bollworm abundance was measured with two complementary methods: checking bolls of non-*Bt* cotton for larvae and capturing male moths in *Bt* cotton fields with pheromone-baited traps.

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Bolls. Pink bollworm abundance in bolls of non-*Bt* cotton plants in Arizona was determined from 1997 to 2009 by sampling bolls from commercial cotton fields during August to November and cutting them open to check for larvae as described previously[14]. The mean sample size per year was 18,200 bolls (range = 2,900–54,300) from 7 to 44 cotton fields.

Pheromone traps. Male pink bollworm moths were captured in *Bt* cotton fields from 1998 to 2009 using delta traps baited with septa impregnated with 4 mg of the pink bollworm female sex pheromone gossyplure (1:1 mixture of the Z,E-7,11 and Z,Z-7,11 isomers of hexadecadienyl acetate, Shin-Etsu Corporation)[43]. Traps were near field edges, usually at 0.8 m high (range = 0.5–1.5 m). Traps and lures were changed every week. Traps were brought into the laboratory where moths were identified under magnification. During the eradication program when sterile moths were released, sterile males in traps were identified visually by the red dye used in their larval diet. When wild pink bollworm males were rare in traps, particularly during 2008 and 2009, pink bollworm males that did not readily show dye were subjected to additional testing as follows: each male was ground individually with a glass rod in a glass shell vial containing several milliliters of acetone. A strip of Whatman no. 4 filter paper trimmed to a point at the top was put in the glass vial. After the acetone rose to the top of the filter paper and evaporated, the male was deemed sterile if red dye appeared at the top of the filter paper and wild if no red dye was visible.

We analyzed data on wild males caught in traps that were collected from the field each year from 15 April to 15 June because data for this period were available for all years from 1998 to 2009. The mean number of traps per year was 10,400 (range = 1,498–29,928) with a seasonal total of up to nine traps (one per week) at each monitoring site. More than 150 *Bt* cotton fields were monitored with traps each year.

A previous analysis based on data from 1992 to 2001 (before the eradication program) showed significant declines in pink bollworm males captured in pheromone traps in regions of Arizona where abundance of *Bt* cotton was high but not in regions of Arizona where abundance of *Bt* cotton was low[21]. Compared with the data reported and analyzed here (**Fig. 3b**), the previous analysis differs in terms of the years studied (1992 to 2001 before; 1998 to 2009 here), the criteria for standardizing the time period examined within years (accumulation of degree-days before; calendar dates here); spatial scale (15 regions of Arizona analyzed separately before; statewide data pooled here), and the distribution of pheromone traps (traps near all cotton fields before; only traps in *Bt* cotton fields here). The analysis here shows a steep statewide decline in males captured in pheromone traps in *Bt* cotton fields during the eradication program (2006 to 2009) but not before the eradication program (1998 to 2005) (**Fig. 3b**).

Insecticide sprays. Insecticide sprays targeting pink bollworm in Arizona cotton from 2006 to 2009 were calculated by adding the number of sprays made by growers and by the ACRPC as part of the eradication program. The ACRPC sprayed when larval infestation reached or exceeded 5% of bolls. Growers sprayed based on their own criteria. The number of sprays made by growers against pink bollworm was estimated as described previously[28]. The mean number of sprays per ha per year made by growers against pink bollworm was 0.068 in 2006, 0.0095 in 2007, 0 in 2008 and 0 in 2009 (ref. 10). The mean number of sprays per ha per year made by the ACRPC against pink bollworm was 0.070 in 2006, 0.0035 in 2007, 0.00029 in 2008 and 0 in 2009. Data for 1996 to 2005, which reflect only sprays made by growers, were reported previously[27].

Analysis of data on pink bollworm abundance and insecticide sprays. We calculated the percentage decrease in abundance as: 100% – [(final abundance/initial abundance) × 100%]. For example, infestation of non-*Bt* cotton bolls was 0.012% in 2009 (final abundance) and 15.3% in 2005 (initial abundance). The percentage decrease in abundance was 100% – [(0.012%/15.3%) × 100%], which equals 99.9%.

We tested for effects of the eradication program on three response variables: (i) infestation of non-*Bt* cotton bolls, (ii) wild males caught per trap per week in *Bt* cotton fields, and (iii) insecticide sprays per ha per year targeting pink bollworm. To test for effects of the eradication program on infestation of non-*Bt* cotton bolls, we compared trends for two time periods: years with *Bt* cotton before the eradication program (1997–2005) and years during the eradication program (2006–2009). For each period, simple linear regression was used to evaluate the association between the percentage of infested bolls (log transformed) and year. We also used covariance analysis to evaluate the effects of year, treatment (before versus during the eradication program), and their interaction on the percentage of infested bolls (log transformed). In this analysis a significant interaction term indicates that the slope before the eradication program differs from the slope during the eradication program. For the covariance analysis of the boll infestation data, years before the eradication program were coded as 1 (1997) to 9 (2005) and during the eradication program as 1 (2006) to 4 (2009). As with the boll infestation data, we used simple linear regression and covariance analysis to evaluate the effects of the eradication program on the number of wild males caught per trap per week in *Bt* cotton fields (log transformed). For this analysis, years with available trap data before the eradication program were 1998–2005 and were coded as 1–8. We used the same approach to analyze the effects of the eradication program on the number of insecticide sprays targeting pink bollworm. Because the spray data included a zero (for 2009), we added 0.0001 to the number of sprays before performing the log transformation[44]. For sprays, years analyzed before the eradication program were 1996 to 2005 and were coded as 1–10. Analyses were performed in JMP[45].

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