Ingenious ways of *Bt* resistance management

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Rational application of insecticides is part of the integrated pest management (IPM). However, excessive application of chemical pesticides has resulted in the development of polygenic insecticide resistance in insect pests[1]. Once insect pests develop resistance to chemical pesticides, management of insect pests becomes more tricky and arduous. It is difficult to prevent insect pests from developing resistance to pesticides. However, a number of time-tested methods are available, which can delay this resistance development and such practices form part of the insecticide resistance management (IRM). These practices include monitoring pest populations, focusing on economic thresholds for insecticide application, applying IPM practices, application of correct, judicious and timely dosages of insecticides, crop rotation, insecticide rotations, protecting beneficial insects and other arthropods, use of biocontrol agents and biopesticides and sterile insect release (SIT), etc. (http://www.irac-online.org).

With the advent of *Bt* transgenic technology globally and in the last decade in India, especially in the form of *Bt* cotton, area and productivity of cotton have increased. However, several pests have evolved resistance to *Bt*, similar to pests developing resistance to chemical pesticides. The threat of cotton's pest, *Helicoverpa armigera* developing resistance to Bt protein is looming large[2–4]. Addressing the *Bt* resistance requires additionally different set of tools and approaches, as compared to conventional IRM. Such specific methods that are available today include high-dose/refugia, mixture of (*Bt*) toxins, gene pyramiding, incomplete insect resistance, increasing fitness costs and use of newer and more potent *Bt* genes[5]. Among these methods, the most effective and simple method is the high-dose/refugia, mandated globally, which aims to render any resistance functionally recessive by using such high *Bt* toxin concentrations that heterozygote-resistant insects do not survive. Refugia broadly refer to the non-transgenic crops that are susceptible to the same insect pests which are unable to cause damage to transgenic counterparts. Refugia are always grown together with their transgenic counterpart crop. They are nearly isogenic to their transgenic crops but lacking the transgene(s). In most cases, pests cannot differentiate refugia and transgenics. Inclusion of refugia along with transgenic crops during cultivation reduces selection pressure operable against the pest since pests can thrive, reproduce, complete life cycle and multiply on safe haven refugia normally. Most of the rare, resistant insects surviving and emerging from *Bt* crops will mate with the relatively abundant susceptible insects arising from adjacent refugia. If resistance is inherited as a recessive trait, as normally reported, the progeny of resistant and susceptible insects, with dominant susceptible genotype, also get killed upon feeding *Bt* crops (http://www.epa.gov/EPA-PEST/1998/January/Day-14/paper.pdf). This iterative step ensues that evolution of resistance is avoided. However, globally and more so in India, stringent compliance of refugia is a major limitation that offsets the whole premise of *Bt* resistance management. This is because farmers seldom use the mandatory refugia seeds supplied along with transgenic seeds as refugia are susceptible to pest damage and are not remunerative. Additionally, minimal insecticide sprays are needed to be taken upon refugia to effectively manage pest populations that may build upon refugia as well as reducing the possibility of multiplication of resistant pest genotypes. Even this step is hardly undertaken by most farmers who may try to save on pesticide expenditures. This further complicates the issue of *Bt* resistance management. Therefore, even though refugia are effective in *Bt* IRM, their use in reality is far from promising. Other complementary methods which are equally effective are hence also needed.

SIT is a method of biological control whereby overwhelming numbers of sterile male insects are generated by irradiation and subsequently released. The sterile males compete with the wild males for mating female insects. If a female mates with a sterile male, it produces no offspring, thus drastically reducing the next generation's population. Repeated releases of sterile male insects can eventually wipe out a population. This technique was successfully used to eradicate the New world screw worm fly (*Cochliomyia hominivorax*) in North America and later on fruit flies, particularly the Medfly (*Ceratitis capitata*) in America and Egypt, the melon fly (*Bactrocera cucurbitae*) in Okinawa, Southern Japan, the Mexican fruit fly (*Anastrepha ludens*) and the Tsetse fly (*Glossina* spp.) that causes sleeping sickness (African Trypanosomiasis) disorder, in Zanzibar. This technique was pioneered in the 1950s by American entomologists, Raymond C. Bushland and Edward F. Knipling, who later jointly received the 1992 World Food Prize.

Recently, Tabashnik *et al.*[6] have successfully attempted in suppressing the development of resistance to *Bt* cotton in a cotton pink bollworm (PBW, *Pectinophora gossypiella*), pest by ingeniously applying SIT, an old but effective entomological method. PBW is one of the world's most destructive and invasive pests of cotton. *Cry1Ac Bt* toxin effectively kills this pest. However, there are many reports globally, including India, of PBW and other pests developing resistance to *Bt*[7–10]. PBW resistance to Cry1Ac Bt protein exemplifies 'Mode 1' high level resistance characterized by recessive inheritance and reduced binding of at least one *Cry1A* toxin and little or no cross resistance to *Cry1C* type of *Bt* toxin[8]. As part of a well coordinated, multitactic and large-scale effort to eradicate the menace of PBW, *P. gossypiella*, from the southwestern United States including Arizona and northern Mexico, the researchers adopted SIT as an alternative strategy, other than refugia. The eradication programme was undertaken during 2006–2009.

The programme included, apart from commercial cultivation of *Bt* cotton:

- Mapping cotton fields for *Bt* crops along with extent of refuge plantations.
- Measuring PBW abundance with two complementary methods, viz. analysing PBW infestation levels on non-*Bt* cotton and estimating the populations by capturing male moths in *Bt* cotton

Table 1. Efficacy of pink bollworm (PBW) eradication programme by SIT[6]

Parameter	Before the programme (up to 2005)	At the end of the programme (2006–2009)	Remarks
Extent of refugia planted	37.4%	~3%	More land for *Bt* cotton and increased yield
Infestation rate on non-*Bt* cotton	15.3%	0.012%	99.9% reduction in infestation
Number of wild male PBW caught per trap per week	26.7	0.0054	99.98% reduction
Mean number of insecticide sprays per hectare per year	2.7	0	Increased profits and associated benefits (cleaner health and environment)
Mean annual cost of yield losses and plant protection	US$ 18 million	US$ 0.17 million	Increased net profits

fields using female sex pheromone traps.

- Monitoring PBW resistance to *Cry1Ac* and *Cry2Ab Bt* toxins by detecting mutant PBW cadherin receptor alleles (linked with *Bt* resistance)[8,11,12].
- Sterile male moth releases – *En masse* release of around 2 billion gamma irradiated and sterilized moths using small airplanes throughout each cotton growing season during the 2006–2009 four-year study. Sterile moth releases were made frequently throughout the season, so that sterile moths were available consistently for mating with wild fertile moths. The mean release rate of sterile PBW during the study was more than 600 times higher than the simulated rate that suppressed recessive resistance to *Bt* cotton for more than 20 years without refuges.
- Cultural methods of quantification of PBW field populations through the use of female sex pheromone traps.
- Enumeration of emergence of resistant genotypes of PBW by insect bioassays (rearing freshly hatched neonate larvae on semisynthetic lab diets infused with different dosages of *Bt* toxin and estimating resistant insects that may survive on *Bt* toxin diets).
- Calculation of application of insecticide sprays targeting PBW.
- Use of a stochastic, spatially explicit model for PBW resistance to *Bt*, which also used Indian data[13].

The effectiveness of the PBW eradication programme was tested by evaluating three response variables, viz. levels of PBW infestation on non-*Bt* cotton bolls, number of wild male moths caught using pheromone traps in *Bt* cotton fields and quantum of insecticide sprays used by farmers during the period of study. Indeed, since the deployment of the eradication programme, there were significant improvements in many parameters like reduction in pest infestation, insecticide sprays, annual expenditure towards insecticides and concurrent yield losses (Table 1). The mean annual expenditure on crop protection also reduced significantly adding profits to farmers.

The following are benefits of SIT:

- By using *Bt* cotton as one component of a comprehensive IPM programme, Arizona farmers also greatly reduced insecticide application against all cotton pests, including those not killed by *Bt* cotton.
- Populations of PBW declined dramatically with corresponding declines in the infestation rates; steep declines in the number of male moths captured by using pheromone traps also corroborated the reduction in pest populations.
- Development of resistance in PBW to *Bt* cotton was suppressed even without refuges. Molecular screening for the three mutations in the *cadherin* gene that are linked with PBW resistance to Cry1Ac Bt protein and insect bioassays did not identify any resistant allele or resistant insect genotype.
- Requirement of refugia declined.
- Local farmers and farming communities widely adopted SIT over large areas.

With so many benefits that SIT can offer, one can easily estimate the magnitude of relevance of this study in Indian context, in the management of the American bollworm (ABW) on (*Bt*) cotton, two points of which are as follows:

- Fortunately, PBW is not a major pest on cotton in India. It is ABW (*H. armigera*) which causes severe damage to cotton productivity and against this pest *Bt* cotton is available in India. However, the model SIT to address PBW in cotton in USA should be easily applicable for ABW in India also albeit with minor modifications. This and other pests cause an annual loss of US$ 300 million in India. Insecticides to the tune of more than US$ 300 million are used mainly on cotton, thus bleeding tremendous national exchequer and causing hazards to health and environment. ABW is a polyphagous pest, recorded from 60 cultivated and 67 wild host plants, but totaling for 161 plants altogether in 49 species. ABW is known to have developed resistance to all major insecticide molecules, with a potential to develop resistance to *Bt* also[2,14–16].
- The *Bt* transgenic technology has therefore made significant developments in India in boosting the quality and productivity of cotton as well as health, environment and cotton farmers' livelihood[17]. Presently *Bt* cotton commercialization in India involves supply of seeds of refugia separately along with seeds of *Bt* cotton. There are always chances in theory and practice that farmers will not sow refugia and grow only *Bt* cotton since cultivation of refugia brings about reduction of productivity to the extent of proportion of refugia in *Bt* cotton fields, resulting in lower compliance of mandatory refugia in India.

Therefore, SIT also provides an opportunity to Indian researchers to augment the power of *Bt* resistance management. Further, apart from adoption of SIT, IRM can be augmented with many innovative options as follows:

- Use of transgenic insects carrying dominant, repressible lethal genetic systems (for example, RIDL approach)[18–20]. This needs committed *de novo* framing of biosafety guidelines in India and other countries, for release of transgenic insects, after comprehensively taking into account the pest biology and ecology.
- Gene pyramiding (use of two or more *Bt* genes and *Bt* with plant resistance genes; use of *cyt* genes; RNAi technology).
- Proposal that *Bt* seeds be mixed together with seeds of refugia instead of packing and selling separately (unstructured refugia).
- Innovative use of refugia; for example, using non-*Bt* crops like corn as a refuge for *Bt* cotton in case of ABW[21,22].
- Regular monitoring for resistance development in insect pests with modelling[23], bioassays, field efficacy tests and molecular screening.
- Incorporation of fitness costs in the deterministic and stochastic models using field data[19,23].
- Application of the knowledge of fitness costs to design better refuges[24].
- Rational integration of other methods of IPM like use of entomopathogen-based biopesticides and natural enemies.

In this 16th year of commercialization of GM crops, many advances have been made and benefits reaped in agricultural biotechnology. India has been a leading developing country with high levels of adoption of *Bt* technology in the form of *Bt* cotton. Nearly 6.3 million farmers grow 9.4 million hectares of *Bt* cotton, equivalent to 86% adoption rate. Insect resistance trait, in the form of *Bt*, was the fastest growing trait in 2009–2010 at 21% growth. This reflects the true definition of sustainable development[17]. At the same time, a caveat that remains is that there are a number of challenges to maintain the usefulness of *Bt* transgenic technology in cotton and forthcoming crops like brinjal, so as to effectively address the problem of development of *Bt* resistance in insects. Ingenious and innovative use of multitactic *Bt* resistance management strategies can definitely go a long way to fully harness the fruits of *Bt* transgenic technology.

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