

COTTON PINK BOLLWORM (*PECTINOPHORA GOSSYPIELLA*) MANAGEMENT WITH THE GOAL OF ERADICATION FROM THE COTTON PRODUCING COUNTRIES OF THE WORLD

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Abstract. Bt-toxin is not effective anymore in controlling cotton pink bollworm in the major cotton producing countries of South Asia. The cultivation of Bt-cotton without the use of 5% non-Bt plants as a refuge crop has made pink bollworm resistant to this toxin. On the other hand this pest has been successfully eradicated from the main cotton growing areas of the United States. The wise use of 5% non-Bt cotton as refugee has provided successful control over this pest in Australia and China. The genetically engineered cotton encoding the Cry1Ac, Cry2Ab, or Cry1F protein does not have a stable insecticidal efficacy. There is fluctuation in its expression during different cotton plant growth stages. Significant reduction in Bt-toxin expression and resistance for pink bollworm has been reported especially near crop maturation. The early crop termination through early maturing varieties, harvest aid chemicals and use of growth regulators can be a key to disrupt pink bollworm diapause phase. Varieties with Bt-toxin along with several morphological and anatomical features that provide natural shelter against bollworm attack should be cultivated in the affected regions. The collective use of integrated pest management approach, sterile PBW moth release, pheromone treatments, crop management for host free period, use of early maturing varieties, natural insect pest resistant varieties, adaptation toward the shortening of crop season, use of 5% refuge crop and transgenic *Bacillus thuringiensis* (Bt) cotton have great impact on the management of this pest of cotton and can change the current suboptimal worse condition of the effected cotton growing areas of the world.

Keywords: cotton chewing insects, Bt-toxin resistance, pink bollworm damage, integrated pest management, pink bollworm invasion

Abbreviations: Bt: *Bacillus thuringiensis*; Cry1Ac, Cry2Ab: crystal insecticidal proteins produced by a bacterium (*Bacillus thuringiensis*); Cry1F: crystalline entomocidal protoxin; CDFA: The California Department of Food and Agriculture; USDA: The United States Department of Agriculture

Introduction

With the introduction of Bt-cotton in South Asia the farmers of the region did not pay attention to the value of refuge crops. Pink bollworm (*Pectinophora gossypiella*) the destructive pest of cotton has developed resistance against Bt-toxin and these nations are facing huge economic losses. This article is all about the background of how this destructive pest developed resistance against Bt-toxin, how developed nations have worked wonder by eliminating this destructive pest from their fields and what measures the currently suffering nations can carry out to eradicate it (Tabashnik et al., 2021). After almost a century of struggle United States cotton is free from the devastating pink bollworm (USDA, 2018). More concerning is the fact that the worm is happily chewing up Bollgard-II and is causing severe cotton production losses of worth millions of

dollars in south Asia (Kranthi, 2011; Ingole et al., 2019). According to an estimate one million bales of cotton is destroyed due to pink bollworm every year in Pakistan (Sayyed et al., 2008). On one side insect resistance to transgenic cotton (Tabashnik et al., 2009; Carrière et al., 2010; Bagla, 2010; Downes et al., 2010; Zhang et al., 2011; Wan et al., 2012; Tabashnik and Carrière, 2010) and pesticides (Schmutter, 1985; Labbe et al., 2005; Levot and Sales, 2008; Sayyed et al., 2008) is proving Darwin's theory of "survival of the fittest" (Tabashnik and Carrière, 2019). On the other hand, world top cotton producing nations either eradicating it like in America, declining its resistance against Bt-cotton like in China or have full control like in Australia (Tabashnik and Carrière, 2019). Invasive life forms when survive and widespread in a new habitat become not only difficult to eradicate but also emerge as a global threat (Pyšek et al., 2020; Martinez et al., 2020 and Simberloff et al., 2020).

The pink bollworm is causing serious economic losses to the cotton crop throughout the world. According to an estimate the destructive pest is causing huge losses with an average range of 3 to 50 per cent in seed cotton yield and oil contents (Anjum et al., 2019). American states of Arizona and California have witnessed a great deal of cotton crop damage after invasion of pink bollworm in 1978. In just a single decade the total cotton crop area dropped from 79,942 ha to 37,130 ha. The total value of cotton dropped approximately \$174,009,389 and the pesticides costs raised to \$264.39/ha. Meanwhile the whitefly populations in the cotton fields also rise to an exponential level resulting in appearance of several disease symptoms and yield losses (Johnson et al., 1982). The year prior to Bt cotton introduction (1995), at a cost of \$373 million and nearly two thirds of cotton crop cultivated land in the United States was treated with insecticides to control cotton pink bollworms (Williams, 1996). Pink bollworm was causing cotton yield damage worth of above 0.25 billions dollar even after the introduction of Bt-cotton in USA (Frisvold and Tronstad, 2003). Each year in America pink bollworm control and yield losses costs cotton producers more than US\$32 million (McGaughan et al., 2017). Before cultivation of Bt-cotton in China it reduced cotton yield by 10% (Cai et al., 1985). In the major cotton growing area of China (Wuhan) the fibre yield losses were estimated about 17-26% (Luo et al., 1986). In the top cotton producing country of the world including Brazil and Egypt it still causes 20% or higher losses in cotton crop (Ismail, 2019). If 1% infestation of pink bollworm in cotton would increase then almost 2.5-6% of cotton yields would decrease in Turkey (Unlu and Bilgic, 2004). In Sudan, the potential yield losses due to pink boll worm infestation could reach up to 10.7% (Darling, 1951). Different surveys results revealed a 20 and 30% yield losses due to widespread infestation of pink bollworm on Bt cotton across the different sites in a range of 40-95% in India (Fand et al., 2019). During 2017-18 there was epidemic level pink bollworm infestation in major cotton growing states of India. The infestation ranged between 8-92% with corresponding yield losses of 10-30% (Anonymous, 2019). It is estimated that in Pakistan, farmers spend US\$300 million on pesticides annually, of which more than 80% is used on cotton, especially for bollworms (Rao, 2007). After 1911, the year of pink bollworm invasion into North America, the cotton growers used different efforts to control its population in the cotton fields. These practices included the use of conventional pesticides, cultivation of modern Bt-cotton varieties, different cultural managements like shredding of cotton residues during winter to kill pink bollworm larvae. The government also planned disrupted mating technique by sterile male dispersal and using sex pheromones etc. Although these efforts resulted in the suppression of pink bollworm population but the

cost for these efforts accounted for a worth of \$32 million per year. This leads to the idea of pink bollworm complete eradication by using all these control methods together by the National Cotton Council (Grefenstette et al., 2009; Marjorie, 2019). The article aimed to review literature discussing methods meant to control cotton pink bollworm, with the aim to investigate the idea of global eradication. Here we identify the problem areas that resulted in pink bollworm resistant to Bt-cotton and the consequences of this negligence. Further, we focused the priority areas helping in its control and eradication. Here we summarize the invasion history and global spread of PBW. Moreover, the impact of collaborative efforts between the governments and different farmer organizations are summarized. Further, the results of different integrated pest management methods in combination with Bt-cotton are discussed.

Pink bollworm invasion history

It is believed that the centre of origin of pink bollworm is Australia or its neighbouring islands (*Fig. 1*), on a malvaceous family plant other than cotton. The positional relationship of Indonesia with the origin is very important in investigating the history of spreader of pink bollworm. As Indonesia is not a cotton producing country therefore it is ignored in the investigations but the earliest record of pink bollworm on Java dates from 1903 (Kalshoven et al., 1981). More recently, the presence of pink bollworm on Bali and its adjacent islands suggested that it is widespread in Indonesia (Burrows et al., 1982). The trade and Hindu expansion is a hypothetical scenario that explains the movement of pink bollworm into the India. From here it reached to the African continent by traders. With the import of American cotton into India, this insect preferred it and quickly shifted its hosts. Seed transport may have resulted in its movement to Egypt another historical cotton growing country, “the land of Nile”. The movement of this destructive pest to South American continent with famous cotton growing areas of Brazil and Mexico occurred through the transport of infested seed originating in Egypt. On the other hand India is considered the centre of spreader to Malaysia, China and Hawaii through the transport of infested seed. Even its spreader is reported to USA during 19 from Indian sub-continent (Brown, 2002; Noble, 1969). Pink bollworm has been described from India in 1843 and subsequently recovered in Hawaii (1901), East Africa (1904), Malaysia and Burma (1906), Egypt (1907), Australia (1911), Mexico (1911), Brazil (1913), Texas (1917), China (1918) and California (1965) (Burrows et al., 1982).

Mechanism of pink bollworm resistance

Transgenic cotton has reduced the need for chemical pesticides. Millions of growers worldwide are cultivating this transgenic cotton. This genetically engineered cotton produces caterpillar-killing proteins from a bacterium source (*Bacillus thuringiensis*) (Bt) (Tabashnik and Carrière, 2019). These pests have developed resistance against this killer protein and reduced its efficacy (*Table 1*). Initially Bt cotton showed perfect results against pink bollworm but with the passage of time the pest undergoes mutations and developed resistance against Bt toxin (Qiu, 2010; Wan et al., 2012; Ojha Sree et al., 2014). In India during 2010-2017 pink bollworm resistance to transgenic cotton (Cry1Ac) and (Cry1Ac + Cry2Ab) have been located in ten major cotton growing states. This study provides the evidence of field evolved resistance of these pests to Bt-I and Bt-II cotton (Naik et al., 2018). These mutations were rare before the commercialization of this

transgenic cotton. However, when two resistant pink bollworm moths get a chance to mate, they produce resistant off-springs. If their population remains unchecked, this increases the proportion of resistant pests in every generation. In a study in central Indian states, pink bollworm resistant samples have been collected from Bt and non-Bt cotton fields. They have observed reduction in labelled Cry1Ac protein binding activity to the receptors membrane of pink bollworm larva showing very high tolerance to Cry1Ac (Lu et al., 2013). They pooled Cry1Ac-resistant strains and feed them on Cry1Ac diet. This pooled sample of resistance pink bollworm again showed significant reduction in protein binding activity. The reduced binding of Cry1Ac to receptors could be anticipated to pink bollworm getting resistant against Bt-toxin and Bollgard hybrids (Ojha et al., 2014). The knowledge of level of dominance for Bt protein resistance in a population is very important because it affects the success of the refuge strategy. This approach of refuge crop is most widely adopted strategy to delay pest resistance development to Bt cotton throughout the world (Zhang et al., 2012).



Figure 1. The history of spreader of pink bollworm. (A) Pink bollworm spreader to the whole world starts from the centre of origin 'Australia' or its neighbouring islands. From here through the way of Indonesia (Java) it reached to India through trade. (B) From India (centre of spreader) it reached to the African continent (Egypt), USA, China and Malaysian islands by traders. (C) From Africa (Egypt) it reached Brazil and Mexico through the transport of infested seed

Refuges can delay resistance

One strategy to maintain progeny of Bt-toxin susceptible moths within the insect population in the cotton field is the use of non-Bt cotton as refuse crop. The normal moth mate with resistance or normal moth the progeny will remain Bt-toxin susceptible. And probability of mating two resistant moths will remain low. Now most of the countries either use refuge crop to maintain Bt-toxin susceptible insect progeny (Wan et al., 2012) or cultivating doubled toxins Bollgard hybrids that would require pink

bollworm to mutate twice in order to survive (Tabashnik et al., 2008). Here we must relate the story of “pink bollworm versus Bt cotton” in the three different areas of the world (Tabashnik and Carrière, 2019). In United States when Bt-cotton was first introduced and planted for cultivation Environmental Protection Agency of the country made it a mandatory requirement to plant 5% of their cotton acreage as non-Bt cotton state-wide. They also dropped billions of sterile pink boll worm moths from airplanes into cotton fields during 2006 (Tabashnik et al., 2010). This was to reduce the probability of Bt-toxin resistance moth to get a little chance of mating with another resistant moth. This would lead to mate a sterile moth to stop reproduction of the fertile offspring. The cultivation of Bt-cotton with the use of 5 per cent non-Bt cotton as refuge, sterile moths, along with other control methods (Lu et al., 2012), eventually led to the eradication of pink bollworm from commercial cotton-growing areas (USDA, 2018). In China the situation was a bit different as pink boll worm was not the major chewing worm in the cotton fields. Instead army worm (*Helicoverpa armigera*) was the main destructive pest which already has the option of other crops to feed unlike the pink bollworm that feed exclusively on cotton. These other crops naturally serve as refuges for army worm (*Helicoverpa armigera*). Meanwhile the pink boll worm population resistant to Bt-toxin begin to increase in the Bt-cotton field in the absent of refuges (Wan et al., 2012). Luckily the cotton seed companies begin to sell second generation Bt-cotton seed mixed with 5% non-Bt-cotton seed and growers were not aware of this refuge mixer. They preferred hybrid seed with second generation of Bt-toxin to boost yield and reduce their production cost. This reverse the situation and pink bollworm resistance to Bt-toxin began to reduce because of increase in refuge (Tabashnik et al., 1990). In India the Bt-cotton seed was sold with a separate packing of non-Bt-cotton seed as refuge. The cotton growers were not properly educated with the importance of refuges and within a time span of less than a decade pink bollworm got resistance against first generation of Bt-toxin. Meanwhile most of the growers switched toward cultivation of second generation of Bt-cotton. Here pink bollworm was already resistant to the first toxin, and resistance to the second toxin evolved rapidly. Now the situation in India is worse as Bollgard hybrids with two Bt-toxins provides little or no control over the devastating pest. The possible way out seems adopting toward shortening the crop season (Tabashnik and Carrière, 2019). The evolution of resistance is real and the only way to coop with it is to slow down this resistance process. And a general principal that can work for any transgenic crop is “refuges can delay resistance” (Shen et al., 2010; Tabashnik and Carrière, 2019; Kranthi et al., 2005).

Fluctuation in Bt-protein expression and varied effectiveness

The Cry toxin crystals get soluble in the insect midgut and bind to the receptors. This damages the mid-gut apical membrane and ultimately death of the insect occurs (Kranthi et al., 2005; Qiong et al., 2013; Jurat-Fuentes and Crickmore, 2017). The genetically engineered Bt-cotton, encoding the Cry1Ac, Cry2Ab, or Cry1F protein, effectively guard against the bollworm damage (Shen et al., 2010 and Levine et al., 2016) however, the insecticidal efficacy is not stable (Liu et al., 2019). The production and maintaining of Bt-toxin expression throughout the Bt-cotton growing season in an adequate and consistent amount in the susceptible plant organs is very important to prevent the harm of targeted pests. However, many studies have reported its fluctuation in expression during different cotton plant growth stages, resulting in varied insecticidal

efficacy (Wan et al., 2005; Chen et al., 2017 and Bravo et al., 2018). Significant reduction in Bt-toxin expression and resistance for *Helicoverpa* spp. was reported especially after flowering (Wu, 2007; Knight and Rogers, 2013; Chen et al., 2017). Near crop maturation significant reduction in Cry1Ac protein expression in the plant leaves was also reported (Wu et al., 2003 and Chen et al., 2004). The Bt-toxin proteins level in second generation Bt-cotton carrying both Cry1Ac and Cry1Ab genes, was reported to be higher during the early plant stages and from anthesis onwards decrease significantly. The Bt-toxin protein resistance against targeted pests dropped below the lethal level of 1.9 µg g⁻¹ and maintained only for 110 days. After this time period pink bollworm can easily harm the cotton crop (Guo et al., 2001; Kranthi et al., 2005).

Table 1. The different aspects of the pink bollworm resistance and their possible solution

Sr. no.	Different aspects of the pink bollworm resistance	References	Possible solutions
1.	The cultivation of Bt-cotton without the use of 5% non-Bt plants	Tabashnik and Carriere, 2019; Wan et al., 2012; Lu et al., 2012; Tabashnik et al., 2010, 2008	Refuge crop can delay PBW resistance development against Bt-cotton
2.	Fluctuation in Bt-protein expression and varied effectiveness	Bravo et al., 2018; Chen et al., 2017; Kranthi et al., 2005; Wan et al., 2005; Guo et al., 2001	Development of new Bt-cotton varieties with standard Bt-protein expression
3.	Bt-cotton varieties with prolong stand in the field (sowing timing early Feb and late maturing) favouring PBW life cycle	Asif et al., 2017; Sarwar, 2017; Reddy et al., 2015; Jatoi et al., 2012; Costa et al., 2011; Qureshi et al., 2009; Yu and Huang, 1990; Michel and Gomez, 1992; Singh et al., 1988	Cultivation of short duration and early maturing varieties
4.	Lack of integrated pest management practices	Tabashnik et al., 2021; Maruti et al., 2020; Marjorie, 2019; Sarwar, 2017; Grefenstette et al., 2009; Arif et al., 2007	Integrated pest management practices especially pheromone application for mating disruption and sterile pink bollworm moth releases
5.	Lack of coordination between government and farming community	Tabashnik et al., 2021; Marjorie, 2019; CDFA, 2018; Grefenstette et al., 2009; Henneberry and Naranjo, 1998	Following example of USDA Area-wide PBW Eradication Program

Mode of action throughout growing season

Depending on environmental conditions, pink bollworms can live and produce up to 1-4 summer generations. The cotton bolls are the favourite and most desirable egg-laying and feeding site for the development of larvae (Saunders, 1843; Beasley and Adams, 1995). The pink bollworm larvae bore into the squares and bolls after hatching from eggs laid by pink bollworm moths and cause rosette blooms. In the cotton bolls, the growing pink bollworm larvae feed and destroy 1-5 seeds before completing development and dropping to the soil for pupation. The lint is also damage by it by cutting it through its mouthparts (Peter et al., 1987). The pink bollworm spends the winter in the larval stage in diapause (Singh et al., 1988; Michel and Gomez, 1992) which is largely photoperiod induced. The larval entrance into the diapause increases from mid of September to the mid of October with an approximate percentage of 5-

90%. The early crop termination can be a key to disrupt this diapause phase and ultimate control of this destructive pest in cotton. Harvest aid chemicals and use of growth regulators (Costa et al., 2011) can also be utilized for the effective destruction of unwanted squares and small bolls which can serve as site of overwintering feeding and shelter for the pink bollworm population. Cutting off irrigation to eliminate the food supply and production of green bolls by the early September can be a good strategy. Immediately after crop termination and harvest, shredding the cotton plants increases the chances of larvae destruction. Unharvested bolls are rapidly dried by shredding the plants and high temperature further destroys larvae. Plough to a depth of at least 6 inches can be very helpful. 50 to 70% flooding in December can reduce populations of overwintering pink bollworms more effective than flooding in November or January (Anonymous, 2019). The cotton crop should be timely sprayed with insecticides until the phase of 3 weeks old last harvestable boll (Henneberry and Naranjo, 1998).

Breeding strategies

The technology behind the eradication of the pink bollworm has four primary components: 1) extensive survey; 2) transgenic Bt cotton; 3) pheromone application for mating disruption; and, 4) sterile pink bollworm moth releases (Tabashnik et al., 2021). The latest breeding strategy to confer resistance in cotton plant against pink bollworm is the genetic engineering for the production of Bt-toxin (Tabashnik and Carrière, 2019; (Kranthi et al., 2005; Qiong et al., 2013; Jurat-Fuentes and Crickmore, 2017). Furthermore, before the introduction of genetically engineered Bt-cotton different morphological and physiological characters of cotton plant in combination of insect behaviour were considered for the development of resistance against this devastating pest of cotton. The feeding preferences of insects are dependent on several morphological and anatomical features of a host plant. The different plant characters like leaf and stem color, Shape of bracts (frego), leaf shape (okera), presence of hairs and spines on leaves, nectaries with deposition of waxes, plant cell wall lignifications, sturdiness of tissue etc., define the fate of insect attack either alone or in combination (Ponti, 1977; Painter, 1951; Din et al., 2016; Silva et al., 2008; Rahman et al., 2013). Different varieties have different resistance level against insect pest attack (Goussain et al., 2005; Pathan, et al., 2007; Inbar and Gerling, 2008; Nawab et al., 2014). Pink bollworm (*Pectinophora gossypiella*) had minimum infestation on certain genotypes with Okra leaves frego bract, red leaf color. These traits alone or in combination are found effective to minimize insect pest attack. These traits should be considered during any breeding program especially for commercial cultivars development (Indrayani and Sumaitini, 2007). **Okra leaf:** Okra leaf type genotypes have less surface area and provide better light penetration and air circulation so are resistant to pink bollworm (Wilson and George, 1982; Wilson, 1986). **Frego Bracts:** In narrow and small bract genotypes the boll are naked and therefore are less damaged as compared to broad and normal bract genotypes. These define the pink bollworm moth preference for oviposition (Baloch et al., 2001; Rahman et al., 2008; Jones et al., 1989; Brazzel and Martin, 1959). **Glabrous and nectariless:** The genotypes with glabrous (hairs and spines on leaves) and nectariless traits show lower bollworm damage due to less preference of moth for oviposition (Jones et al., 1989; Brazzel and Martin, 1959). **Red leaf color:** Pink bollworm had minimum infestation on certain genotypes with, red leaf color and is found effective to restrict insect pest population (Indrayani and Sumaitini,

2007). **Early maturity:** Controlling the number of overwintering pink bollworms by reduced their food supply later in the year through the use of early maturing varieties and plant growth regulators (Jatoi et al., 2012; Kairon and Singh, 1996; Beasley and Adams, 1995).

In certain studies backcross breeding techniques is utilized to transfer these morphological traits to the next generations to develop resistant plant material. The genetic analysis verified these traits under additive genetic control with significantly high narrow sense heritability estimates and genotype $\times$ environment interactions were not significant for these traits. Therefore, it should not be difficult to transfer these resistance traits into desirable backgrounds (Wilson and George, 1983). Although Bt-cotton provide resistance against chewing insects but the evolution of resistance against this toxin in the insect pest is an alarming situation. In china another breeding technique gave promising results in eleven year field study which involves crossing Bt-cotton with conventional non-Bt cotton and then sowing the second generation. This strategy yields a random mixture within fields of three-quarters of plants that produce Bt protein and one-quarter that does not. This outcome illustrates that non-Bt plants in a seed mixture can boost survival of susceptible insects and delay resistance (Wan et al., 2017). Another breeding strategy to increase the insect resistance of cotton plants would be to combine the Bt-toxin trait with other plant morphological traits resistance against insects and pest traits such as nectariless, okra leaf, and early maturity, known to reduce pink bollworm and other pest insects (Douglas et al., 1992). Early maturing cotton varieties are desirable for a number of the reasons as they require relatively less inputs like fertilizer and irrigation and also uses less labour. Thus, early maturing varieties provide comparatively increased economic returns on account of reduced cost of inputs and crop management. Besides, early maturing cottons are exposed to unfavourable environmental conditions for a relatively shorter period, hence provides an escape to late season pest attack, especially boll worm pest complex. Short season cottons are also of immense importance in the countries where sequence of other crops succeed cotton crop, thus fitting very well in cropping pattern of wheat followed by cotton crop for sustainable agriculture (Jatoi et al., 2012). A number of indicators are proposed that determine earliness in cotton are reduction in monopodia, profuse flowering, higher boll setting and opening at earlier stages of crop growth, less and smaller leaves, short internode length, semi-determinate types, lower sympodial node number, short sympodial branches, cluster fruit bearing types, medium boll size, sub-okra types and dwarf stature plants (Kairon and Singh, 1996; Baloch and Baloch, 2004). Short-season upland cotton (*Gossypium hirsutum* L.), which is also called early-maturing cotton, generally exhibits a dwarf, compact phenotype with fewer leaves, shorter internodes and fruiting branches, and shorter growth period than middle-late-maturing cotton (Yu and Huang, 1990). Making use of the markers linked closely to QTL for early-maturing traits for MAS is an effective method for the simultaneous improvement of early maturity and other properties in cotton (Chengqi et al., 2013). The majority of yield in cotton plant is produced from the first and second fruiting sites on main-stem nodes 9 through 14. As much as 80% of the yield originates at these sites (Costa et al., 2011) Because of these growth characteristics, ways to modify and control the flowering/fruiting of the cotton plants are often desirable. This encourages the management practices to terminate crop at this stage to have maximum economic yield benefits along with suppressing overwintering insect population. The other option is the use of plant growth regulators (PGRs).

A success story

The managerial activities have successfully prevented incipient infestations of PBW from becoming established in the cotton growing areas of California. A cooperative integrated pest control program by the cotton growers of the California and the United States Department of Agriculture (USDA) has been in continual operation since 1967. The major funding contributors are the cotton growers themselves and USDA share is nearly about 8% (CDFA, 2018). The success of this program is because of the collective use of the previous researches all together like integrated pest management approach, sterile PBW moth release (Tabashnik et al., 2021; Staten and Walters, 2020; Leonard et al., 2020) host free period including crop destruction and plant back restrictions, pheromone treatments and 100% transgenic *Bacillus thuringiensis* (Bt) cotton in the region to keep pink bollworm infestations below economic impact levels. In this program no pesticides were applied instead sterile PBW moths were utilized. They were produced at the CDFA/USDA pink bollworm rearing facilities in Phoenix, Arizona. The pheromone-baited insect traps were used to inspect weekly from April through October in order to know the proper time and stage to release sterile moths. Different areas were targeted and the sterile pink bollworm moths were released by aircrafts on daily bases. After successful reduction of pink bollworm the release of sterile moths ended in 2012. This helped in reduction of millions of pounds of pesticides which cost by \$100- \$150 per acre (CDFA, 2018). This program also used pheromones to disrupt the moths' mating activities. With a collaborative efforts of the local agriculture departments the cultural controls activities were applied by managing a "host-free" period through identification favourable of planting and crop destruction periods. The identification of favourable planting and crop destruction periods was monitored. These were carried out through collaboration between the local agriculture departments and farming community. Complete shredding of cotton stalks and the dislodging of cotton plant roots to prevent regrowth were encouraged. These all activities resulted in successful completion of this integrated pest control program. In addition to these activities the 95% cotton growing area is under Bt-cotton cultivation which is in compliance with the USDA Area-wide PBW Eradication Program (Henneberry and Naranjo, 1998). Although area-wide application of these methods resulted in its suppression but it accounted for at least the costs of \$32 million per year. As a result, the National Cotton Council proposed its eradication by using all of these control methods together (Grefenstette et al., 2009; Marjorie, 2019).

Conclusions

The reason behind pink boll worm resistance to Bt-toxin in the main cotton growing areas of South Asia can be significant reduction in its expression near the crop maturation. The early crop termination through early maturing varieties, harvest aid chemicals and use of growth regulators can be a key to disrupt pink bollworm diapause phase. The cultivation of Bt-cotton with the strict use of 5% non-Bt as refuge and timely utilization of different integrated pest management practices is essential for management of pink bollworm in the cotton crop. A collaborative international revolution against this pest is needed as a pest in one part of the world can be in any part of the world.

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