

## Resistance in Pink Bollworm *Pectinophora gossypiella* (Saunders) against Bt cotton, a major threat to cotton in India: A brief review

### Abstract

The pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) is a primary pest of cotton in many regions of the world. In many parts of India, pink bollworm (PBW) has become resistant to BG-I (Bollgard I®) that expresses a single Bt gene (Cry1Ac) in 2009 and BG-II (Cry1Ac and cry 2Ab) in 2015. The brief review addresses the possible reasons and solutions to resistance posed by this insect. The review addresses the following points: reasons for resistance in PBW is discussed; the life-history is discussed, and its distinctiveness how it is different from other bollworms; the introduction of hybrid cotton leaded to insecticide treadmill in India; reduced accomplishment of the refugia recommendations also contributed to the problem; poor compliance of the bio-safety laws also led ad to the problem; unfavorable crop management practices prove to be an add-on to the problem. Furthermore, the possible solutions of the problem are discussed; monitoring and mating disruption; cultivation of High-density short season (HD-SS) pure line varieties; case studies of sterile insect technique (SIT) proved to be supporting in control of PBW; RNA interference technique can overcome the problem of resistance; use of chemical insecticides in different manner can change the perspective; proper cultural or farming practices can make the difference; Bio-control agents can overcome the problem; extension functionaries can help in solving the PBW problem. Therefore, the management of resistance in PBW requires a broader vision and development of technology.

**Keywords:** Cotton, Bollworm, Hybrid, Resistance

### 1. Introduction:

Stepping back, the Genetic Engineering Appraisal Committee (GEAC) had approved the first transgenic single-gene *Bt*-cotton hybrids (Bollgard I®) in 2002 and the next-generation cotton transgenic with stacked *Bt* genes (Bollgard II®) called 'pyramids' in 2006 in India. The introduction of Bt cotton, between 2002 and 2006, reduced the use of insecticides from 40,672 tons<sup>1</sup> to the tune of 9,000 t<sup>2</sup>. Genetically engineered (GE) crops had proved to be potent tools of Integrated Pest Management (IPM) programs and important factor in improving the sustainability, economics, and social interactions among the growers<sup>3,4</sup>, thus resulted into increase in area under GE crops since 1996, reaching 190 million hectares in 2016 globally<sup>5</sup>.

Though Bt cotton proved to be a huge success, the sustainable use of this technique, need thorough

35 understanding of the GE trait introduced, properties of the target crop, the cropping pattern and the socio  
36 economic standards are important for the successful integration of the GE crops into IPM systems<sup>6</sup>. One of  
37 the biggest challenges for sustainable use of technology is the evolution of resistance. The major reason  
38 behind the evolution of resistance is over-reliance on Bt crops without appropriate adoption of the  
39 Insect Resistance Management (IRM) or IPM practices<sup>7,8</sup>. One such example was resistance to Bollgard- I  
40 (Cry1Ac) in cotton against the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lep.: Gelechiidae),  
41 in India<sup>9</sup> and Bollgard II in 2015. Meissle<sup>10</sup> (2016) mentioned that GE crops should not be considered as  
42 the only solution to control pest. GE traits should complement a broader IPM strategy filled with a  
43 companion and compatible selective tactics, but should not remain the central focus for all the pest  
44 challenges in the system. It is equally crucial that other IPM practices are developed, optimized, and  
45 maintained for all crop pests<sup>11</sup>. In this paper, we highlighted the possible reasons for the development of  
46 resistance in PBW and the challenges faced by the cotton industry. We also explained different IPM  
47 practices adopted in different cotton-growing countries all over the world based on the case studies and  
48 how they can be useful in Indian cotton production systems.

## 49 2. Possible reasons for resistance in Pink Bollworm

50 The resistance problem in cotton against Pink Bollworm is not attributed to a single factor, but it is an  
51 amalgamation of multiple factors. Following are the possible reasons for ~~the resistance~~-resistance: -

### 52 2.1 Life history

53 Life history theory is a very central and necessary part of both population ecology and general  
54 evolutionary theory, and it is especially useful in pest forecasting and management<sup>12</sup>. Identification of the  
55 pest, understanding its biology and seasonal population trends, damaging life stages and their habitats,  
56 nature of the damage and its economic significance, the vulnerability of each life stage for one or more  
57 control options, host preference, and alternate hosts, predictability of pest occurrence based on the  
58 environment, cropping trends, farming practices, and other influencing factors, and all the related  
59 information is critical for identifying an effective control strategy<sup>13</sup>.

60 Early in the cotton season, PBW eggs are laid in the sheltered places of the plant axis of petioles  
61 or peduncles, the underside of young leaves, on buds or flowers but once the bolls are 15 days old, these  
62 are the most favorable sites for oviposition. The incubation period is 3-6 days. The larval cycle lasts for 9-  
63 14 days in hotter regions. The mature larvae are either 'short-cycle' or 'long cycle' differing according to  
64 the state of diapause. Short cycle larvae form a tunnel in the cuticle and fall to the ground by cutting a  
65 round exit hole through the carpel wall, leaving it as a transparent window and pupate inside the  
66 ground. Pupation takes place inside a loose cocoon with a highly webbed exit at one end. The pupal period  
67 ranges between 8 and 13 days. The life cycle is completed in 3-6 weeks. The late-season has invariably

#### Comment [K1]:

-Authors could tighten up the introductory section. It looks too brief and simple and acceptable for review studies or synthesis report. They could consider doing so with research progress, general overview of the key terms or subjects, the need for this review study to some key stakeholders or players, implications or effects of the phenomenon on some key sectors like agriculture, health and so on.

**-Re-organization or structuring of entire manuscript:** The introduction must be followed by an empirical review or analytical framework/model (**Methodology/Approach**) which should be designed by the authors to link up how the entire study is set up, how the various sections were examined or analyzed. Hence, a section for "**Methodology**" must be created to briefly inform readers how authors analyzed data (i.e., **was data analyzed using a systematic literature review [SLR]?**), acquired information and research strategy used in drafting this review report.

-After doing the above, authors can then present the results/discuss findings against existing body of knowledge. These sections: 2.0, 2.1 to 2.5-should be placed under is section for consistency, clarity and coherence.

-Again, authors could consider coining each of these sections in a format that encapsulates: general descriptions of the subject, overview/gaps and opportunities in these multiple factors that result in resistance or drive this study's objectives.

68 overlapping broods. On the other hand, the long cycle larvae enter diapause and spin up a spherical cells  
 69 which is tough thick-walled, closely woven, referred to as "hibernaculum" with no exit hole. The long-  
 70 term larvae always occur during the end of the crop season, where there are mature bolls present and  
 71 larvae often form their hibernacula inside seeds. Hibernacula may occupy single seeds or double seeds. *P.*  
 72 *gossypiella* hibernate as full-fed larvae during cold weather. Diapause larvae often spin up in the lint of  
 73 an open boll and if still active in ginnery, will spin up on bales of lint, bags of seed, or in cracks and  
 74 crevices. Therefore, the long-lived larvae act as a source of inoculums and are more harmful. The PBW  
 75 life-cycle differences than other bollworms could be the reason for early resistance in this pest:-

76 A. The effective population of PBW buildup starts after 100 to 110 days of crop emergence, while the  
 77 peak infestations occur after 140 days, which coincides with the harvest of the crop<sup>14</sup>. The cry toxin  
 78 expression levels in leaves decline after 110-120 days after sowing. Therefore, Bt-cotton controls  
 79 bollworms effectively at 90-100% up to 100-110 days after sowing and 70-80% of the bollworm  
 80 larvae thereafter. The reduction of Bt protein content in late-season cotton could be due to the over-  
 81 expression of the Bt gene at earlier stages, which leads to gene regulation at post-transcription levels  
 82 and consequently results in gene silencing at a later stage<sup>9,15-17</sup>.

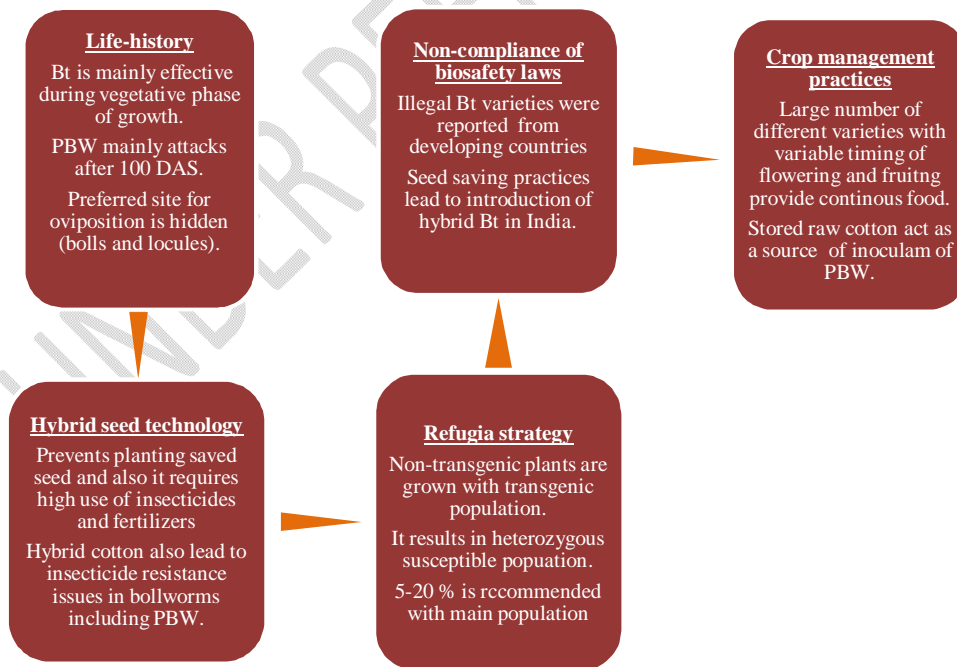


Figure 1:- Reasons of resistance in pink bollworm.

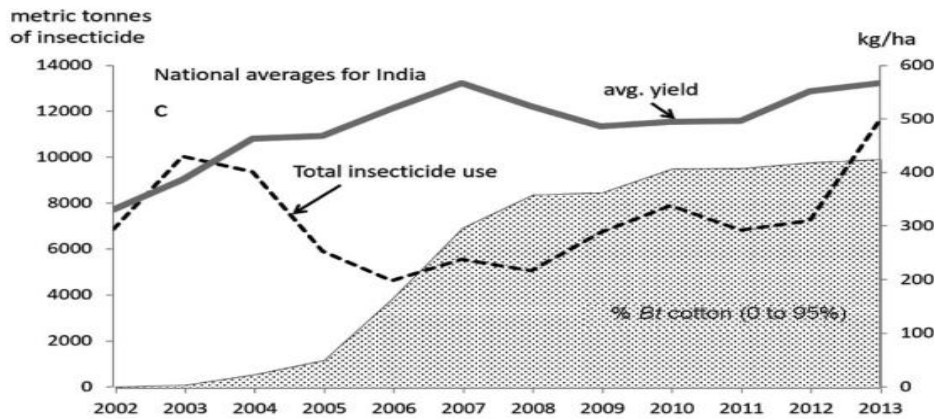


Figure 2: A summary of average national yield, insecticide use, and Bt cotton adoption in India (Gutierrez et al.,<sup>19</sup> 2017).

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- 90 B. The preferred site for oviposition of PBW is bolls and locules where they are well protected and
- 91 remain alive for many months, whereas *H.armigera* and *E.vitella* lay eggs on leaves. The bolls on F-
- 92 1 plants contain seeds that segregate in a 3:1 ratio of Bt: non-Bt. Therefore bollworm larvae can
- 93 survive on the 25% non-Bt seeds in green bolls. The pink bollworm survival in Bt-cotton is mainly
- 94 due to the presence of such segregating Bt-cotton seeds in the green bolls of the Bt-cotton F1
- 95 hybrids<sup>18</sup>.
- 96 C. As discussed earlier, late-season larvae enter in diapause, and incidence of *P. gossypiella* during the
- 97 season commences from the moth emerging from the overwintering larvae through the summer
- 98 season. This situation can be avoided by using high-density short duration varieties. Survival of the
- 99 pest from one season to another is entirely through hibernating larvae in seeds, soils, and plant debris.

100 **2.2 Hybrid cotton leads to pesticide treadmill in India**

101 Cotton production in India has a 5000-year history but large changes started in 1790 when New  
102 World cotton (chiefly *Gossypium hirsutum* L. and later *Gossypium barbadense* L.) were introduced by the  
103 colonial British to feed their developing industrial revolution<sup>20</sup>. It's always been a debatable topic, why  
104 hybrid cotton was only introduced to India in the 1970s<sup>21</sup>, but nowhere in the world, when it prevents  
105 planting a saved seed, besides it required a high use of insecticides and fertilizers<sup>22,23</sup>. The whole  
106 world grows highly fertile pure line varieties of *G.hirsutum*. The hybrid Bt cotton introduction provides  
107 initial relief to the farmers, however, the insecticides usage reached pre-2002 levels by 2013, as Bt cotton  
108 induced the outbreaks of new pests (e.g., plant bugs, whiteflies, mealybugs). Also, the introduction  
109 of hybrid cotton in India, ushered to the chain of problems, as excessive use of insecticides created wide  
110 ecological disruption and outbreaks of secondary pests that lead to yield losses and insecticide resistance  
111 issues in many defoliators including pink bollworms and other bollworms in the 1990s<sup>9</sup>, hence  
112 complicated the insecticide-based cotton production system in India<sup>24,25</sup>. By 2013, the area under Bt  
113 cotton reached up to 95 percent. By this time, the pure line varieties disappeared from the market<sup>21</sup> and  
114 farmers got trapped in new hybrid technology treadmills<sup>26</sup>.

115 **2.3 Refugia strategy**

116 Refugia strategy mainly focuses on the use of the biotechnological aspect of plant protection  
117 management, where an area consisting of non-transgenic plants is grown with a transgenic population,  
118 that supports sufficient homozygous susceptible insects to mate with the majority of homozygous  
119 resistant individuals, resulting in heterozygous susceptible progeny. Retrospective analyses of global  
120 resistance monitoring data lead to the assumption that refuges can substantially delay resistance to Bt  
121 crops<sup>27-30</sup>. The effectiveness of refuge strategy is governed by two key conditions: sufficient refuges of  
122 non-Bt host plants and a toxin concentration in Bt plants, that can kill all hybrid progeny, which was also  
123 called as 'high dose' criterion<sup>31</sup>. Andow and Hutchison<sup>32</sup> (1998) stated that the high-dose refuge strategy  
124 demands that Bt plants express a sufficiently high concentration of Bt proteins so that 95% of the  
125 heterozygous individuals carrying one copy of a major resistance allele can be killed. 'Based on data, the  
126 US EPA Scientific Advisory Panel (SAP) on Bt Plant-Pesticides and Resistance  
127 Management<sup>33,34</sup> suggested that a working definition of the high dose should be 'a dose 25 times the toxin  
128 concentration needed to kill Bt-susceptible larvae.' According to the researchers, higher pink bollworm  
129 resistance is noticed in India and China as compared to the developed countries. Pink bollworm resistance  
130 to Bt cotton has been reported in the field in India, where farmer compliance with the refuge strategy has  
131 been low<sup>9,35</sup>. Wan *et al*<sup>36</sup> (2012) hypothesized that lower concentration of Cry1Ac in Bt cotton in these  
132 countries compared with the United States could be the reason for the acceleration of pink bollworm

133 resistance in India and China, that could lead to an increase in survival of heterozygotes and thus increase  
134 the dominance of resistance.

135 Earlier recommendations of non-transgenic crops specified that between 5 and 20% of any given  
136 area should be included as refugia for Bt-cotton<sup>37-39</sup>. Ministry of Environment, Forest and Climate  
137 Change, Government of India (GoI) had recommended sowing of 20% of the area with non-Bt cotton as a  
138 'structured' refuge for both types of Bt-cotton<sup>40</sup>, but compliance is very low. Indian Agriculture Research  
139 Institute (IARI) had recommended 'refuge-in-bag' (RIB) with 95:5 (90-95% Bt seeds: 5-10% non-Bt seeds  
140 as permissible limits) and the non-Bt seeds must be of the near-isogenic hybrid corresponding to the BG-  
141 II hybrid<sup>40</sup>.

142 To address the problem of insect resistance, Insect Resistance Management (IRM) programs have  
143 been proactively implemented wherever Bt crops have been commercialized, with these programs being  
144 mandatory in some countries including the USA, Canada, Australia, the EU, the Philippines, and South  
145 Africa<sup>41</sup>. The Australian cotton industry showcased one great story for the adoption of IRM. In the 1990s,  
146 Australian cotton growers were challenged with a high level of Lepidopteran resistance to insecticides,  
147 which almost led to the end of the cotton industry<sup>42-44</sup>. High awareness of the need for IRM by growers  
148 and appropriate education and training has resulted in refuge adoption that is consistent near to 100% in  
149 Australia.

150 Presently Bt cotton commercialization in India involves the supply of seeds of refugia separately  
151 along with seeds of Bt cotton. There are always changes in theory and practice that farmers will not sow  
152 refugia and grow only Bt cotton since the cultivation of refugia brings about a reduction of productivity to  
153 the extent of proportion of refugia in Bt cotton fields, resulting in lower compliance of mandatory refugia  
154 in India.

#### 155 **2.4 Poor compliance of Intellectual Property Rights (IPR) recommendations**

156 Biosafety regulations can also have unintended consequences. Between 2002 and 2006, only one  
157 company in India – MAHYCO Monsanto Biotech (MMB) – got permission to sell the Bt gene implanted  
158 in cotton, and therefore, regulations in effect gave MMB a monopoly on the sale of legal Bt. However,  
159 farmers planted Bt cotton in India before the official approval, in 2002<sup>45</sup>. Despite the abundant resources  
160 and time invested in promulgating new laws and setting up new institutions for biosafety, illegal  
161 transgenic varieties were reported from many developing countries such as Brazil, China, and India<sup>46-48</sup>. A  
162 survey of 200 cotton farmers found that 60% of their cotton area in 2007 was under illegal Bt seeds<sup>49</sup>.  
163 Monsanto speculated that CryI Ac concentration was lower in the unapproved Bt cotton than approved Bt  
164 cotton in India and that early use of unapproved Bt cotton ushered to the resistance problem.

165 The failure to enforce bio-safety laws is widespread and demands explanation. In India, farmers  
166 exhibit a tradition of seed saving, seed exchange, and seed experimentation that has historically produced

167 better crops and better incomes. Authors like Herring<sup>45</sup> (2007) and Shah<sup>50</sup> (2005) have emphasized the  
168 limits of legal monopolies in seeds and suggest that farmers are empowered to make "gray market"  
169 versions of the legal seed. Many scientists have also suggested that non-compliance of seed laws by the  
170 farmers could be the reason why Bt is only introduced in hybrid seeds in India.

### 171 **2.5 Crop management practices**

172 Many crop management practices lead to an increase in the incidence of PBW. When cotton  
173 prices are hiked, farmers extend their crop up to April-May and this practice can provide continuous  
174 availability of cotton all through the year. The minor seasonal peak of PBW occurs in June-July and it  
175 coincides with the early (April-May) sown cotton crop at the time of flowering. As PBW is a winter pest,  
176 it mainly causes damage in November, which can be prevented. In the absence of the crop or the crop  
177 residues, the pupae enter in diapauses in December. However, if the crop is available beyond November,  
178 the pest continues to survive on the fruiting parts. This extended phase intensifies Bt-toxin selection  
179 pressure and resistance development is accelerated. Long term storage of raw cotton in ginning mills and  
180 market yards serve as a source of PBW inoculum to the ensuing crop<sup>18</sup>.

## 181 **3. Strategies for resistance management**

### 182 **3.1 Monitoring**

183 The Mating Disruption (MD) technique works with the principle that the air in an agricultural  
184 field (e.g. orchard) is saturated with sex pheromone, which prevents male pests from locating females  
185 and thereby preventing the reproduction process<sup>51</sup>. This technique has been widely used successfully to  
186 control lepidopteran species. MD technique is widely used for the management of PBW<sup>52</sup>. In the case of  
187 pink bollworm, female release sex pheromone called gossyplure<sup>53</sup> and this pheromone has been used for  
188 monitoring and mating disruption studies to provide comparatively better control from insecticides<sup>54-56</sup>.  
189 Kranthi<sup>18</sup> (2015) recommended the use of 'pheromone traps' and 'green boll dissection' for regular  
190 monitoring and mating disruption at the rate of 8 moths per trap per night or 10% damage in green bolls.  
191 Boguslawski and Basedow<sup>57</sup> (2001) used the MD technique on cotton for PBW in a semi-arid region of  
192 Egypt and claimed that it was 52% more efficient than conventional methods. Lykouressis *et al*<sup>58</sup> (2005)  
193 reported that this technique was more effective in preventing damage by PBW if applied early in the  
194 cotton-growing season. Special care should be taken to monitor the emergence of moths in and around  
195 the ginneries with the help of pheromone traps to confirm the possibilities of presence of PBW larvae  
196 and subsequent moth emergence.

### 197 **3.2 High-density short-season varieties**

198 The solution to the insecticide treadmill and to prevent ecological hazard in Indian irrigated and  
199 rainfed cotton is short-season high-density non-hybrid non-GMO cotton with minimal insecticide

**Comment [K2]:** Description or evaluation for some of the sub-sections in section 3.0 are too loose. From an objective/constructive perspective, the authors need to make thorough assessments from their perspectives briefly, based on other proposed strategies. It must be made clear which strategies are standardized or globally accepted, under which conditions or parameters, possible limitations for the implementation of some of these strategies, linking them up to factors raised in previous sections.

200 use<sup>21,26</sup> the potential of which has been demonstrated by Indian scientists at the Central Institute for Cotton  
 201 Research, Nagpur<sup>59</sup>. The manipulation of row spacing, plant density, and the spatial arrangements of  
 202 cotton plants, for obtaining higher yield has been attempted by agronomists for several decades in many  
 203 countries. The concept of high-density cotton planting, more popularly known as Ultra Narrow Row  
 204 (UNR) cotton was introduced by Briggs *et al*<sup>60</sup>(1967). UNR cotton has row spacing as low as 20 cm and  
 205 plant population ranges from 2 to 2.5 lacs plants/ha, compared to conventional cotton where rows are 90  
 206 to 100 cm apart and have a plant population of about 100,000 plants/ha. Kranthi<sup>61</sup>(2012) visited Brazil,  
 207 studied pure-line high-density short-season (HD-SS) grown there, and made the following observations:

- 208 • Like India, a very large area of cotton in Brazil is under rain-fed conditions and HD-SS was  
 209 found perfectly suitable under these conditions.
- 210 • Compact sympodial varieties were cultivated in Brazil which was suitable for high-density  
 211 planting geometry. High-density planting used the specification of 90X10 cm and 76X10 cm and  
 212 45X10 cm spacing was used in ultra-narrow-row planting.
- 213 • Higher productivity in Brazil was achieved through the development of compact monopodial  
 214 (sympodial) varieties<sup>59</sup>.

215 Based on this study, scientists at the Central Institute for Cotton Research (CICR), Nagpur, also developed  
 216 a pure line high yielding non-Bt HD-SS rain-fed varieties of *G. hirsutum* and *G. arboreum*. The *G.*  
 217 *hirsutum* pure line non-Bt HD-SS variety PKV-081 produced an average of 1944 kg of seed cotton/ha at  
 218 16 plants m<sup>-2</sup>, whereas the pure line non-Bt HD-SS *G. arboreum* variety CINA-404 yielded an average of  
 219 1,973 kg/ha at 22 plants m<sup>-2</sup>(table 1). Seed cotton yields in the two non-Bt rain-fed kinds of cotton were  
 220 about half those in irrigated cotton in southern California, but they were about 2.2 times the current  
 221 average yield of long season Bt hybrids in Maharashtra. In spite of the effects of the rainfall on the yields,  
 222 the HD-SS have the ability to better utilize the rainfall thereby reduce the yield variability<sup>62,63</sup>. Equally  
 223 important, the HD-SS varieties were found to escape the PBW attack, since they germinate in mid-June  
 224 coinciding with the monsoon rains when adult emergence from overwintering pupae has occurred. Also,  
 225 the short season length of fewer than 150 days was found unsuitable for the development of the PBW  
 226 population.

227 Table: 1 Data on pure line non-Bt HD-SS varieties (kg seed cotton/ha): data reproduced from  
 228 Venugopalan *et al*<sup>62</sup>(2011).

Plants/ha	Anjali	PKV-81	CCH-724	CNH120MB	NISC-50
<i>G.hirsutum</i> - kg/ha seed cotton					
55000	502	1200	679	1030	1056
111000	847	1714	843	976	890

111000	853	1418	681	1138	1103
166000	966	1921	864	1250	1016
166000	796	1967	835	1289	1052
Plants/ha	CINA-404	PA-255	AKA-07	JK-5	PA-08
<i>G.arboreum</i> - kg/ha seed cotton					
111000	1430	1259	1163	1223	1090
166000	1550	1595	1349	1452	1318
166000	1610	1349	1456	1151	1455
222000	2173	1625	1815	1842	1509
222000	1772	1226	1419	1734	1479

229 NB: Plants m<sup>-2</sup> = plants/ha/10,000.

### 230 3.3 Sterile insect technique (SIT)

231 As early as 1937, E. F. Knipling had conceived an approach to insect control in which the natural  
 232 reproductive processes of the screwworm fly was disrupted by chemical or physical mechanisms, thus  
 233 rendering the insect sterile<sup>64</sup>. Sterile insects are released into the environment in very large numbers (10 to  
 234 100 times the number of native insects) to mate with the native insects that are present in the  
 235 environment. A native female that mates with a sterile male will produce infertile eggs. Since there are 10  
 236 to 100 times more sterile insects in the population than native insects, most of the crosses become sterile.  
 237 As the process is repeated, the number of native insect decreases and the ratio of sterile to native insects  
 238 increase, thus driving the native population to extinction<sup>65</sup>.

239 A sterile moth release program was initiated in 1968 to exclude pink bollworm from cotton in the Central  
 240 Valley of California<sup>66</sup>. The male moths were irradiated (via gamma radiation), sterilized and reared in  
 241 thousands, and released periodically over cotton fields with the help of airplanes. These irradiated males  
 242 would compete with the native males, thus mating with sterilized male prevents egg hatching or the  
 243 produced offspring are sterile<sup>67,68</sup>. Van Steenwyket *al*<sup>69</sup> (1979) reported that mass-reared sterilized males  
 244 were less competitive than their native counterparts, while mass-reared sterilized PBW females were equal  
 245 to or more competitive than native females. However, he also indicated that the combined release of both  
 246 male and female PBW provided a sterile population that was as competitive as native males and females  
 247 in mating ability.

248 A similar multi-tactic eradication program was also launched in Arizona for four years (2006-  
 249 2009) to delay pink bollworm resistance to Bt cotton<sup>70</sup>. Special emphasis was given on the number of  
 250 sterile insects released and the frequency of release, thus the release rate of sterile PBW was more than  
 251 600 times higher than the simulated rate, which resulted in suppression of resistance to Bt cotton for more  
 252 than 20 years without refuges. The US Environmental Protection Agency (EPA) reviewed the proposed  
 253 eradication program<sup>71</sup> and based on the results, allowed the Arizona cotton growers to plant up to 100% Bt

254 cotton producing either one toxin (Cry1Ac) or two toxins (Cry1Ac and Cry2Ab). This resulted in the  
255 dramatic decline of pink bollworm populations in Arizona since the eradication program began in 2006.

256 With the abundant benefits that SIT can offer, it seems that this technique is perfectly relevant in  
257 the Indian context, in the management of the pink bollworm and other bollworms. But Indian conditions  
258 have many problems when it comes to the application of such techniques since the farmer holdings are  
259 very small and the application of these techniques becomes complicated. Therefore, the application of this  
260 technique requires the revision of some government policies and the assistance from the farmers.

261

### 262 3.4 RNA interference

263 The discovery of RNA interference (RNAi) constitutes an important milestone in the study  
264 of regulatory RNAs<sup>72</sup>. In this process, small (s)RNA molecules of 18–31 nucleotides(nt) long effectuate a  
265 sequence-specific gene silencing response, acts at the post-transcriptional level through cleavage or  
266 blockage of longer RNAs containing a matching sequence<sup>73</sup>. The RNAi technique has been thoroughly  
267 researched in the Western corn rootworm (WCR) *D. virgifera virgifera*<sup>74-76</sup>. Baum *et al*<sup>74</sup> (2007) genetically  
268 engineered a transgenic corn crop, to express dsRNA against the V-ATPase. When insect feed on the  
269 modified plant of *D. virgifera virgifera*, larvae get stunted and premature death of the insect take place.  
270 The results were encouraging as a crop protectant due to less feeding damage<sup>74</sup>. Based on the fact, first  
271 RNAi-based insecticide was approved by the United States Environmental Protection Agency (EPA).  
272 This plant-incorporated protectant (PIP) employed stacking of different type of genes in a single host:  
273 dsRNA coupled with different type of Bt-proteins, also targeted the WCR Snf7 gene and was expressed in  
274 the plant<sup>77</sup>. The gene, Snf7 also works as a protein trafficker and when it was regulated, it resulted in  
275 mortality of the insect<sup>78</sup>. This strategy has very less chances of development of resistance due to the  
276 diversified genes used in it<sup>77</sup>. In cotton crop, same study was done against the cotton bollworm  
277 *Helicoverpa armigera* where, the plant-mediated expression of dsRNA targeted the cytochrome P450  
278 monooxygenase gene (CYP6AE14) that could increase the toxic effects of gossypol, a cotton metabolite  
279 that is otherwise tolerated by the cotton bollworm<sup>79</sup>. The silencing of CYP6AE14 led to delayed larval  
280 growth when gossypol was supplemented in the diet<sup>79</sup>. On the lines of *H. armigera*, these studies can also  
281 be conducted for PBW.

### 282 3.5 Chemical insecticides

283 Different surveys conducted by CICR In Gujrat, revealed that various chemical insecticides supported  
284 the growth of PBW population, especially the mixture of two insecticides i.e mixture of monocrotophos +  
285 acephate, when sprayed 3-4 times during early stages of the Bt-cotton crop, leads to reversal of  
286 reproductive to vegetative phase; emergence of fresh green leaves and delays maturity of the crop.  
287 Continuous application (3-4 times) of this combination results in staggered flowering and fruiting. Since

288 flowers remain for much longer period than normal, they can attract bollworms, therefore continuous  
289 maintenance of pink bollworm inoculum takes place in such fields<sup>18</sup>. Therefore, use of such combination  
290 of insecticides should be avoided. Infestation of pink bollworm was high in the open bolls and green bolls  
291 of second picking in such fields. Survey conducted by CICR also revealed that quinalphos or thiodicarb,  
292 type of insecticides should be used in earlier stages and use of synthetic pyrethroids should be used after  
293 October at economic threshold levels of damage since the use of synthetic pyrethroids in earlier stages  
294 will lead to whitefly population outbreak. Wherever farmers had sprayed synthetic pyrethroids in late  
295 October or early November, pink bollworm infestation was negligible. Selection of hybrids that are  
296 sucking pest resistant also helps in control of PBW population, since it supports in avoiding the  
297 application of chemicals such as monocrotophos, acephate, thiomethoxam, acetamiprid, imidacloprid or  
298 clothianidin<sup>80</sup>.

### 299 **3.6 Farming practices**

300 Cultural control plays crucial role in reducing the carryover of PBW to the next season. Therefore,  
301 essential practical measures should be taken to prevent the spread of PBW in the field, which includes  
302 pre-planting, post-harvest and off-season measures<sup>81</sup>. Practices such as removing of cotton stubbles after  
303 the cotton crop season, timely termination of the crop, avoid stacking of cotton stalks for fuel purpose  
304 over long periods and deep summer ploughing to expose the pupae of the surviving larvae are the major  
305 post-harvest season cultural practices. Pre-planting practices i.e selecting timely and early maturing  
306 varieties, drying seeds for 6-8 hours under sun and delinting seeds before sowing is the practice which can  
307 decrease the PBW incidence to some extent<sup>18</sup>. Sowing time also play key role in the incidence and extent  
308 of damage done by the pest. In north India, the sowing time range is narrow, i.e. from 15 April to May  
309 whereas south and central India have staggered sowing, varying from April (under irrigated condition) to  
310 July (under rainfed condition), thus providing continuous influx of source plant for thriving of PBW<sup>82</sup>. Also  
311 the care should be taken that the long duration storage of raw cotton in ginning mills and market yards is  
312 avoided because that can serve as a source of pink bollworms to the ensuing crop. In central India,  
313 wherever irrigation facilities are available, farmers maintain ratoon, which can increase the PBW  
314 incidence<sup>83</sup>.

### 315 **3.7 Bio-control agents**

316 Several genera of Ichneumonids, Braconidae and Trichogrammatidae found attacking PBW. Apanteles,  
317 Bracon and Chelonus are the genera of family Braconidae that have been contributed in management of  
318 PBW. Inundative release of many parasitoids was done in Arizona between the time periods of 1969-78,  
319 however the best performance was achieved by egg- larval parasitoids *Chelonus* spp. (Braconidae).  
320 Legner and Medved (1979)<sup>84</sup> reported that *Chelonus* sp. nr. *curvimaculatus* (Cameron) gave 69.9 %  
321 reduction in infested bolls by PBW in northwestern Australia bollworm larvae under field conditions.

322 Several predaceous orders attack PBW such as Dermoptera, Coleoptera, Hemiptera and Neuroptera.  
323 Predators mainly attack eggs because they exposed more as compared to larvae and pupae<sup>85,86</sup>. The  
324 predator belonging to dermaptera, *Labidura riparia* (Pallas) can attack all the immature stages of PBW  
325 along with pupa<sup>86</sup>. Coleopteran predators mainly attack early instar larvae and eggs. According to  
326 Orphanides *et al.*, (1971)<sup>86</sup>, *Chrysoperla carnea* is the only neuropteran that attack PBW in California.  
327 Steinernematid (Rhabditida: Steinernematidae) are the nematodes which act as obligate insect  
328 parasites<sup>87</sup> and they are associated with a symbiotic bacteria, *Xenorhabdus* spp.<sup>88</sup>. The nematodes enter the  
329 insect body from the inhabitant soil and bacteria are released in insect haemocoel that cause septicemia,  
330 leading to the death of the insect<sup>89</sup>. The nematodes may pass through several generations, and once host  
331 reserves are depleted a new generation of infective juveniles exit the cadaver<sup>90</sup>. *S. carpocapsae* and *S.*  
332 *riobravis* are found very useful in management of diapausing PBW larvae. Entomopathogenic nematodes  
333 have positive affinity towards the other beneficial insects and do not hamper the application of most  
334 chemical fertilizers and insecticides<sup>91,92</sup>.

### 335 3.8 Role of extension functionaries

336 Extension functionaries play crucial role in disseminating the knowledge among the farmers because they  
337 are directly connected to them. Therefore, adoption of survey and surveillance techniques, resistance  
338 monitoring studies and pest forecasting services carried out under IRM programme help farmers in  
339 making the decision regarding the pest i.e. Central Institute of Cotton research (CICR) issues weekly  
340 advisories in nine local languages and English in the CICR web site ([http://www.cicr.org.in/  
341 weekly\\_advisory.htm](http://www.cicr.org.in/weekly_advisory.htm)). Very good initiative is taken by CICR where weekly advisories (E-Kapas) are  
342 sent to 11,893 farmers in Gujarat and 1,80,000 farmers across India through voice mail. CICR project  
343 staff conducts IRM campaign at various field sites (150 sites) across Gujrat. All India Coordinated Crop  
344 Improvement Project (AICRIP) on cotton also involves Front-Line demonstrations (FLDs) for farmers<sup>18</sup>.

### 345 Conclusion

346 In a country like India, where farmers are indulged in many problems like lack of resources, small farm  
347 holdings, illiteracy, poverty, a slower rate of mechanization and, vague government policies, among  
348 others etc., they are incapable of handling such problems at their level. Therefore, it becomes important to  
349 find some serious solution to this problem and the government requires to interfere and invest in research  
350 and development (R & D) methods before it's too late so that the situation doesn't get worse as in case of  
351 American bollworm (*H. armigera*).

### 352 References

#### Comment [K3]: Conclusion:

-The conclusion is too brief. It must clearly highlight the main aim of the study, summary of key findings, relevance of the outcome of this study or review study to researchers, and possible limitations of the study that could suggest or drive future research.  
-Authors could recommend or propose measures that could come before the conclusion section, based on the findings of the study.

- 353 1. Bambawale, O.M. and Jayakumar, P., In: International Conference on Emerging Trends in Production  
354 Processing and Utilisation of Natural Fibres. Mayfair, Worli, Mumbai, India. 2009, **1**, 155–161.
- 355 2. ISAAA. 2009. Biotech Crops in India: the Dawn of a New Era. ISAAA Brief No. 39. ISAAA South Asia  
356 Office, New Delhi, India. p. 34.
- 357 3. Ervin, D. and Jussaume, R., Integrating social science into managing herbicide-resistant weeds and  
358 associated environmental impacts. *Weed Sci.*, 2014, **62**, 403–414. doi: 10.1614/WS-D-13-00085.
- 359 4. Ervin, D. E. and Frisvold, G. B., Community-based approaches to herbicide-resistant weed management:  
360 Lessons from science and practice. *Weed Sci.*, 2016, **64**, 602–626. doi: 10.1614/WS-D-15-00122.
- 361 5. ISAAA. 2017. Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption  
362 Surges as Economic Benefits Accumulate in 22 Years. ISAAA Brief No. 53. ISAAA: Ithaca, NY.
- 363 6. Meissle, M., How to assess the role of genetically engineered crops in integrated plant production? IOBC-  
364 WPRS Bull., 2016, **114**, 23–29.
- 365 7. Gassmann, A. J., Maxwell, P. J. L., Clifton, E. H., Dunbar, M. W., Hoffmann, A. M. and Ingber, D. A.,  
366 Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic  
367 maize. *Proc. Natl. Acad. Sci.*, 2014, **111**, 5141–5146. doi: 10.1073/pnas.1317179111
- 368 8. Tabashnik, B. and Carrière, Y., Surge in insect resistance to transgenic crops and prospects for  
369 sustainability. *Nat. Biotechnol.* 2017, **35**, 926–935 doi:10.1038/nbt.3974
- 370 9. Dhurua, S., and Gujar, G. T., Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm,  
371 *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. *Pest Manag. Sci.*, 2011, **67**,  
372 898–903. doi: 10.1002/ps.2127
- 373 10. Meissle, M., How to assess the role of genetically engineered crops in integrated plant production? IOBC-  
374 WPRS Bull., 2016, **114**, 23–29.
- 375 11. Anderson, J. A., Ellsworth, P. C., Faria, J. C., Head, G.P., Owen, M. D. K., Pilcher, C. D., Shelton, A. M.  
376 and Meissle, M., Genetically Engineered Crops: Importance of Diversified Integrated Pest Management for  
377 Agricultural Sustainability. *Frontiers in Bioengineering and Biotechnology*, 2019, **7**, 24.
- 378 12. SörenNylin, Life history perspectives on pest insects: What's the use?. *Austral Ecol.*, 2001, **26** (6), 507-517
- 379 13. Dara, S.K., The New Integrated Pest Management Paradigm for the Modern Age. *J. Integr. Pest Manag.*,  
380 2019, **10**, (1), 12, <https://doi.org/10.1093/jipm/pmz010>
- 381 14. Henneberry, T.J. and Jech, L.F., Seasonal pink bollworm, *Pectinophora gossypiella* (Saunders), infestation  
382 of transgenic and non-transgenic cottons. *South Western Entomology*, 2000, **25**, 273-286
- 383 15. Olsen, K.M., Daly, J.C., Holt, H.E. and Finnegan, E.J., Season-long variation in expression of cry1Ac gene  
384 and efficacy of *Bacillus thuringiensis* toxin in transgenic cotton against *Helicoverpa armigera* (Lepidoptera:  
385 Noctuidae). *J. Econ. Entomol.*, 2005, **98**(3), 1007–1017. PMID: 16022333
- 386 16. Kranthi, K.R., Naïdu, S., Dhawad, C.S., Tatwawadi, A., Mate, K. and Patil, E. Temporal and intra-plant  
387 variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm,  
388 *Helicoverpa armigera* (Hübner) (Noctuidae: Lepidoptera). *Curr. Sci.* 2005; 89(2): 291–298.
- 389 17. Dong,H.Z., Li, W.J. and Tang, W., Heterosis in yield, endotoxin expression and some physiological  
390 parameters in Bt transgenic cotton. *Plant Breed.*, 2007, **126**,169– 75.
- 391 18. Kranthi, K. R., Cotton Statistics and News, 2015, pp. 1–3.
- 392 19. Gutierrez, A. P., Ponti, L. and Baumgartner, J., A critique on the paper 'Agricultural biotechnology and  
393 crop productivity: macrolevel evidences on contribution of Bt cotton in India'. *Curr. Sci.*, 2017, **112**, 690–  
394 693; <http://www.currentscience.ac.in/Volumes/112/04/0690.pdf>
- 395 20. Beckert, S., Empire of Cotton: A Global History. *The American Historical Review*, 2016, **121** (1), 189–19.
- 396 21. Kranthi, K.R., Cotton production systems-Need for a change in India, *Cotton Statistics and News*, 2014, pp.  
397 4-7.
- 398 22. Basu, A. K. and Paroda, R. S., Hybrid Cotton in India: A Success Story, Asia-Pacific Association of  
399 Agricultural Research Institutions, FAO Regional Office for Asia & the Pacific, Bangkok, Thailand, 1995.
- 400 23. Prasad, C. S., Suicide Deaths and Quality of Indian Cotton: Perspectives from History of Technology and  
401 Khadi Movement. *Econ. Polit. Wkly*, 1999, 34, 12–21.

- 402 24. Kranthi, K. R., Jadhav, D. R., Kranthi, S., Wanjari, R. R., Ali, S. S. and Russell, D. A., Insecticide  
403 resistance in five major insect pests of cotton in India. J. Crop Prot., 2002, **21**, 449–460.
- 404 25. Kranthi, K. R., Russell, D., Wanjari, R., Kherde, M., Munje, S., Lavhe, N., &Armes, N., Inseason changes  
405 in resistance to insecticides in *Helicoverpaarmigera* (Lepidoptera: Noctuidae) in India. J. of Econ.  
406 Entomol., 2002, **95(1)**: 134–142.
- 407 26. Gutierrez, A.P., Ponti, L., Herren, H.R., Baumgartner, J. and Kenmore, P.E., Deconstructing Indian cotton:  
408 weather, yields, and suicides. Environ. Sci. Eur., 2015, **27**, 12
- 409 27. Tabashnik, B.E., Gassmann, A.J., Crowder, D.W. and Carrière, Y. Insect resistance to Bt crops: evidence  
410 versus theory. Nat Biotechnol, 2008, **26**, 199-202
- 411 28. Tabashnik, B.E., Van Rensburg, J.B. and Carrière, Y., Field-evolved insect resistance to Bt crops:  
412 definition, theory and data. J. Econ. Entomol., 2009, **102**, 2011-25
- 413 29. Carrière, Y., Crowder, D.W. and Tabashnik, B.E., Evolutionary ecology of insect adaptation to Bt crops.  
414 Evol Appl., 2010, **3**, 561–573.
- 415 30. Huang, F., Andow, D.A. and Buschman, L.L., Success of the high-dose/refuge resistance management  
416 strategy after 15 years of Bt crop use in North America. Entomol. Exp. Appl., 2011, **140**, 1-16;  
417 <http://dx.doi.org/10.1111/j.1570.7458.2011.01138>.
- 418 31. Gould, F. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu.  
419 Rev. Entomol., 1998, **43**: 701–726.
- 420 32. Andow, D.A. and Hutchison, W.D., Bt corn resistance management. Now or Never: Serious New Plans to  
421 Save a Natural Pest Control (ed. by Union of Concerned Scientists). 1998, Two Brattle Square, Cambridge,  
422 MA, USA. 19-66.
- 423 33. US EPA-SAP (Scientific Advisory Panel) (1998) Report of Subpanel on *Bacillus thuringiensis* (Bt) Plant-  
424 Pesticides and Resistance Management. EPA SAP Report. Available at: [http://](http://www.mindfully.org/GE/FIFRA-SAP-Bt.htm)  
425 [www.mindfully.org/GE/FIFRA-SAP-Bt.htm](http://www.mindfully.org/GE/FIFRA-SAP-Bt.htm)
- 426 34. US EPA (United States Environmental Protection Agency) (2001) Biopesticides Registration Action  
427 Document-*Bacillus thuringiensis* Plant-Incorporated Protectants. E: Benefit Assessment. Available at:  
428 [http://www.epa.gov/oppbpd1/biopesticides/pips/Bt\\_brad2/5-benefits.pdf](http://www.epa.gov/oppbpd1/biopesticides/pips/Bt_brad2/5-benefits.pdf).
- 429 35. Stone, G., Biotechnology and the political ecology of information in India. Hum. Organ., 2004, **63**:127–40.
- 430 36. Wan, P., Huang, Y., Wu, H., Huang, M., Cong, S. and Tabashnik, B.E., Increased frequency of pink  
431 bollworm resistance to Bt toxin Cry1Ac in China. PLoS One., 2012, **7**, 29975; PMID:22238687;  
432 <http://dx.doi.org/10.1371/journal.pone.0029975>.
- 433 37. Anderson, J. L., Letters to Bt corn 12/20/99. United States Environmental Protection Agency (EPA). 2000.  
434 [http://www.epa.gov/oppbpd1/biopesticidesotherdocs/bt\\_position\\_paper\\_618\\_old.htm](http://www.epa.gov/oppbpd1/biopesticidesotherdocs/bt_position_paper_618_old.htm)
- 435 38. Hargrove, T. R., Wrangling over refugia. Am. Sci., 1999, **87**: 24–25.
- 436 39. Shelton, A. M., Tang, J. D., Roush, R. T., Metz, T. D., and Earle, E. D., Field tests on managing resistance  
437 to Bt-engineered plants. Nat. Biotechnology., 2000, **18**, 339–342.
- 438 40. Mohan, K. S. and Sadananda, A. R., Success of refuge-in-bag for *Bt*-cotton hinges on good stewardship.  
439 Curr. Sci., 2019, **117**, 5.
- 440 41. sMatten S. R., Head G. P. and Quemada H. D., How governmental regulation can help or hinder the  
441 integration of Bt crops within IPM programs, in Integration of Insect-Resistant Genetically Modified Crops  
442 Within IPM Programs, eds Romeis J., Shelton A. M., Kennedy G. G., editors. (Dordrecht: Springer), 2008,  
443 27–39. 10.1007/978-1-4020-8373-02
- 444 42. Roush, R., Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed  
445 where pesticide mixtures have not? Philos. Trans. R. Soc. Lond. B Biol. Sci., 1998, **353**, 1777–1786.  
446 10.1098/rstb.1998.0330
- 447 43. Fitt G. P., Deployment and impact of transgenic Bt cotton in Australia, in The Economic and  
448 Environmental Impacts of Agbiotech, ed Kalaitzandonakes N., editor. (New York, NY: Springer), 2003,  
449 141–164. 10.1007/978-1-4615-0177-0\_8

- 450 44. Wilson L. J., Whitehouse M. E. and Herron G. A., The management of insect pests in Australian cotton: an  
451 evolving story. *Annu. Rev. Entomol.*, 2018, **63**, 215–237. 10.1146/annurev-ento-020117-043432
- 452 45. Herring, R. J., Stealth Seeds: Biosafety, Bioproperty, Biopolitics. *J. Dev. Stud.*, 2007, **43** (1), 130-157
- 453 46. Da Silveira, J.M.F.J. and Borges, I. D. C. (2007). Brazil: Confronting the Challenges of Global  
454 Competition and Protecting Biodiversity. *The Gene Revolution*, (pp 104-129). London: Earthscan.
- 455 47. Huang, B., Jin, L. & Liu, J. Molecular cloning and functional characterization of a DREB1/CBF-like gene  
456 (*GhDREB1L*) from cotton. *Sci China Ser C* **50**, 7–14 (2007). <https://doi.org/10.1007/s11427-007-0010-8>.
- 457 48. Ramaswami, B., and Pray, C. (2007). India: Confronting the Challenge – The Potential of Genetically  
458 Modified Crops for the Poor. In S. Fukuda-Parr (Ed.) *The Gene Revolution*, (pp 156-174). London:  
459 Earthscan.
- 460 49. Lalitha, N., Ramaswami, B. and Viswanathan, P. K., India's Experience with Bt Cotton: Case Studies from  
461 Gujarat and Maharashtra. In R. Tripp, (Ed.) *Biotechnology and Agricultural Development: Transgenic  
462 Cotton, Rural Institutions and Resource-Poor Farmers*, 2009, London: Routledge
- 463 50. Shah, E., Local and Global Elite Join Hands: Development and Diffusion of Genetically Modified Bt  
464 Cotton Technology in Gujarat, *Economic and Political Weekly*, 2005, XL (43), pp. 4629- 4640
- 465 51. Ahmed, S.B. and Pfeiffer, D.G., Establishing a mating disruption block in an orchard or vineyard, 2010  
466 <http://www.virginiafruit.ento.vt.edu/MD Bull.html>.
- 467 52. Carde, R.T., Minks, A.K., Control of moth pests by mating disruption: successes and constraints. *Annu.  
468 Rev. Entomol.*, 1995, **40**, 559–585.
- 469 53. Hummel, H.E., Gaston, L.K., Shorey, H.H., Kaae, R.S., Byrne, K.J. & Silverstein, R.M., Clarification of  
470 the chemical status of the pink bollworm sex pheromone. *Science*, 1973, **181**, 873–875.
- 471 54. Baker, T., Staten, R. & Flint, H., Use of pink bollworm pheromone in the Southwestern United States.  
472 *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*  
473 (eds R.L. Ridgway, R.M. Silverstein & M.N. Inscoe), 1990, pp. 417–436. Marcel Dekker, NY.
- 474 55. Flint, H.M., Smith, R.L., Bariola, L.A., Horn, B.R., Forey, D.E. & Kuhn, S.J., Pink bollworm: trap tests  
475 with gossypure. *J. Econ. Entomol.*, 1976, **69**, 535–538.
- 476 56. Gaston, L.K., Kaae, R.S., Shorey, H.H. & Sellers, D., Controlling the pink bollworm by disrupting sex  
477 pheromone communication between adult moths. *Science*, 1977, **196**, 904–905.
- 478 57. Boguslawski, C.V., Basedow, T., Studies in cotton fields in Egypt on the effects of pheromone mating  
479 disruption on *Pectinophora gossypiella* (Saund.) (Lep., Gelechiidae), on the occurrence of other arthropods,  
480 and on yields. *J. Appl. Entomol.*, 2001, **125**, 327–332.
- 481 58. Lykouressis, D., Perdakis, D., Samartzis, D., Fantinou, A. and Toutouzas, S., Management of the pink  
482 bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) by mating disruption in cotton  
483 fields. *Crop Prot.*, 2005, **24**, 177- 183.
- 484 59. Venugopalan, M.V., Kranthi, K.R., Blaise, D., Lakde, S. and Shankaranarayanan, K., High density planting  
485 system in cotton-The Brazil Experience and Indian Initiatives. *Cotton Res. J.*, 2014, **5**(2), 172-185
- 486 60. Briggs, R.E., Patterson, L.L. and Massey, G.D., Within and between-row spacing of cotton. *Arizona  
487 Annual Report. Univ. of Arizona Agric. Ext. Service, Arizona*, 1967, 6-7
- 488 61. Kranthi, K. R., *Bt Cotton: Questions and Answers*, Indian Society for Cotton Improvement, Mumbai, 2012.
- 489 62. Venugopalan, M. V., Prakash, A. H., Kranthi, K. R., Deshmukh, R., Yadav, M. S. and Tandulkar, N. R., In  
490 *World Cotton Research Conference , International Cotton Advisory Committee, Mumbai*, 2011, pp. 341–  
491 346.
- 492 63. Chu C. C. *et al.*, Reduction of pink bollworm (Lepidoptera: Gelechiidae) populations in the Imperial  
493 Valley, California, following mandatory short-season cotton management systems. *J. Econ. Entomol.*,  
494 1996, **89**, 175–182.
- 495 64. Knipling, E. F. 1985. Sterile insect technique as a screwworm control measure: The concept and its  
496 development, pp. 4-7. In O. H. Graham [ed.], *Symposium on Eradication of the Screwworm from the  
497 United States and Mexico. Misc. Publ. Entomol. Soc. America* 62, College Park, MD.

- 498 65. Knipling, E. F., 1979. The Basic Principles of Insect Population Suppression and Management. U. S. Dept.  
499 of Agriculture. Agriculture Handbook No. 512. Washington,
- 500 66. Henneberry, T.J., Pink bollworm sterile moth releases: Suppression of established infestations and  
501 exclusion from noninfested areas. Fruit Flies and the Sterile Insect Techniques (ed. C.O. Calkins, W.  
502 Klassen & P. Liedo), 1994, 181–207. CRC Press, Inc., Boca Raton, FL.
- 503 67. Graham, H.M., Ouye, M.T., Garcia, R.D. and De La Rosa, H.H., Dosages of gamma irradiation for full and  
504 inherited sterility in adult pink bollworms. J. Econ. Entomol., 1972, **65**, 645–650.
- 505 68. Flint, H.M., Staten, R.T., Bariola, L.A. and Palmer, D.L., Gamma-irradiated pink bollworms:  
506 attractiveness, mating, and longevity of females. Environ. Entomol., 1973, **2**, 97–100.
- 507 69. Van Steenwyk, R.A., Henneberry, T.J., Ballmer, G.R., Wolf, W.W. and Sevacherian, V., Mating  
508 competitiveness of laboratory-cultured and sterilized PBW for use in a sterile moth release program. J.  
509 Econ. Entomol., 1979, (**72**): 50
- 510 70. Tabashnik, B.E., Sisterson, M.S., Ellsworth, P.C., Dennehy, T.J., Antilla, L. and Liesner, L., Suppressing  
511 resistance to Bt cotton with sterile insect releases. Nat. Biotechnol. 2010,**28**, 1304-7; PMID:21057498;  
512 <http://dx.doi.org/10.1038/nbt.1704>Arabunisa, M. A. (2014). FDI in retail sector impact on farmers,  
513 agriculture and agri business International journal of economic and businessreview vol. **2**(5).
- 514 71. US EPA October 24–26, 2006: Evaluation of the Resistance Risks from Using 100% Bollgard and Bollgard  
515 II Cotton as Part of a Pink Bollworm Eradication Program in the State of Arizona.  
516 [http://www.epa.gov/scipoly/sap/meetings/2006/102406\\_mtg.htm](http://www.epa.gov/scipoly/sap/meetings/2006/102406_mtg.htm) 4
- 517 72. Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., and Mello, C. C.(1998). Potent and  
518 specific genetic interference by double-stranded RNA in*Caenorhabditis elegans*. Nature 391, 806–811. doi:  
519 10.1038/35888
- 520 73. Siomi, H., and Siomi, M. C. (2009). On the road to reading the RNA-interferencecode. Nature 457, 396–  
521 404. doi: 10.1038/nature07754
- 522 74. Baum, J.A., Bogaert, T., Clinton, W., Heck, G.R., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G.,  
523 Munyikwa, T., Pleau, M., Vaughn, T. and Roberts, J., Control of coleopteran insect pests through RNA  
524 interference. Nat. Biotechnol., 2007, **25**, 1322-1326.
- 525 75. Rangasamy, M. and Siegfried, B. D., Validation of RNA interference inwestern corn rootworm  
526 *DiabroticavirgiferavirgiferaleConte* (Coleoptera: chrysomelidae) adults. Pest Manag. Sci., 2012, **68**, 587–  
527 591. doi: 10.1002/ps.2301
- 528 76. Wu, K., Camargo, C., Fishilevich, E., Narva, K. E., Chen, X. and Taylor, C. E., Distinct fitness costs  
529 associated with the knockdown of RNAi pathway genes in western corn rootworm adults. PLoS One, 2017,  
530 **12**, e0190208. doi: 10.1371/journal.pone.0190208
- 531 77. Head, G. P., Carroll, M. W., Evans, S. P., Rule, D. M., Willse, A. R. and Clark, T. L., Evaluation of  
532 SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy  
533 and resistance management. Pest Manag. Sci., 2017, **73**, 1883–1899. doi: 10.1002/ps.4554
- 534 78. Bolognesi, R., Ramaseshadri, P., Anderson, J., Bachman, P., Clinton, W. and Flannagan, R. Characterizing  
535 the mechanism of action of doublestranded RNA activity against Western Corn Rootworm  
536 (*DiabroticavirgiferavirgiferaleConte*). PLoS One, 2012, **7**, e47534. doi: 10.1371/journal.pone.0047534
- 537 79. Mao, Y.B., Cai, W.J., Wang, J.W., Hong, G.J., Tao, X.Y. and Wang, L.J., Silencing a cotton bollworm  
538 P450 monooxygenase gene by plantmediated RNAi impairs larval tolerance of gossypol. Nat. Biotechnol.,  
539 2007, **25**,1307–1313. doi: 10.1038/nbt1352
- 540 80. Kranthi, K. R., Bt-cotton: questions and answers. Indian Society for Cotton Improvement, Mumbai, 2012,  
541 p. 70; [http://www.cicr.org.in/pdf/Bt book Kranthi.pdf](http://www.cicr.org.in/pdf/Bt%20book%20Kranthi.pdf)
- 542 81. Vennila, S., Biradar, V. K., Sabesh, M. and Bambawale, O. M., Know your cotton insect pest: pink  
543 bollworm. ICAR-Central Institute for Cotton Research, Nagpur, 2007.
- 544 82. Anon., Causes of low yield of cotton during cotton crop season 2015. A report of the Committee  
545 constituted by Agriculture Department, Government of Punjab, Pakistan, University of Agriculture,  
546 Faisalabad, 2016.

- 547 83. Kumar, R., Monga, D., Naik , V.C.B., Singh, P. and V. N. Waghmare,V.N, Incipient infestations and threat  
548 of pink bollworm *Pectinophora gossypiella* (Saunders) on Bollgard-II cotton in the northern cotton-growing  
549 zone of India. Curr. Sci., 2020, **118(9)**, 1454-56.
- 550 84. Legner, E.F and Medved, R.A., Influence of parasitic Hymenoptera on the regulation of pink bollworm,  
551 *Pectinophora gossypiella*, on cotton in the lower Colorado Desert. Environ. Ent., 1979, **8**, 922-30.
- 552 85. Irwin, M.E., Gill, R.W. and Gonzalez, D., Field-cage studies of native egg predators of the pink bollworm  
553 in southern California. J.Econ.Ent., 1974, **67**, 193-96.
- 554 86. Orphanides, G.M., Gonzalez, D. and Bartlett, B.R., Identification and evaluation of pink bollworm  
555 predators in southern California. J.Econ.Ent., 1971, **64**, 421-24
- 556 87. Poinar, G. O., Nematodes for biological control of insects. CRC Press, Boca Raton, FL 1979.
- 557 88. Akhurst, R. J. and Boemare, N. E., Biology and taxonomy of *Xenorhabdus*. In Entomopathogenic  
558 nematodes in biological control. R., Gaugler, and H.K., Kaya, eds. CRC Press, Boca Raton, FL, 1990, 75 -  
559 90.
- 560 89. Kaya, H. K. and Gaugler, R., Entomopathogenic nematodes. Ann. Rev. Entomol., 1993, **38**, 181 -206.
- 561 90. Kung, S. P., Gaugler, R. and Kaya, H.K., Effects of soil temperature, moisture, and relative humidity on  
562 entomopathogenic nematode persistence. J. Invertebr. Pathol., 1991, **57**, 242 -249.
- 563 91. Georgis, R., Kaya, H. K. and Gaugler, R., Effect of steinernematid and heterorhabditid nematodes  
564 (Rhabditida: Steinernematidae: Heterorhabditidae) on non -target arthropods. Environ. Entomol., 1991, **20**,  
565 815 -822.
- 566 92. Poinar, G. O. Jr., Non - insect hosts for the entomogenous rhabditoid nematodes Neoaplectana  
567 (Steinernematidae) and Heterorhabditis (Heterorhabditidae). Rev. Nematol., 1989, 12, 423 -428.

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