Chapter

Tritrophic Association between Bt Cotton, Arthropod Pest and Natural Enemies

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Abstract

Benefits and harmful effects of Bt adoption technology are mainly related with cotton production where lot of insecticides are needed for management of arthropod herbivory and possible negative impact of crystalline Bt protein on parasitoids and predators is real. Therefore, current review information was focused that Bt should be selective for natural enemies and information was collected from different sources especially CAB abstracts as well as citations from many review articles and books. Usefulness of integrated pest management was highlighted with updated literature to cover the contents.

Keywords: biosafety, transgenic crop, beneficial fauna, non-target arthropod, parasitoid and non Bt refugia

1. Introduction

Development of transgenic crops is by incorporation of Bt genes that are very useful for the effective control of lepidopterous larvae [1]. Bt crops include maize, cotton, tobacco, eggplant, tomato, potato, canola, cabbage, soybean, cabbage and broccoli [2–5]. Cultivated area of biotech crops have also been increased to (2 billion hectares in 2015) that was 67.7 million during 2003 [6, 7]. Biological activity of Bt against target insect pest succeeds as the most effective pest management strategy that can be integrated into pest management module of an economic crop [8–10]. Bt crops have environmentally advantage over microbial Bt without its economic drawbacks because they use sun light energy to manufacture toxin and they are available to manage herbivorous arthropod pest throughout the season in the plant [11]. They are also useful because their toxin is active only against a narrow range of target pest as compared with conventional broad-spectrum insecticides (**Table 1**).

1.1 Detection of Bt protein

It depends mainly upon the toxin level produced by a plant of specific cultivar. However, Bt expression is fluctuated with respect to plant part and the growing stage of the crop [13, 14]. At flowering stage an abrupt change in Bt protein has been recorded [15]. Physiological changes in Bt concentration also occur when plants are damaged [16]. Secondary metabolites alter the effectiveness of Bt protein;

Toxin	Year	Bollworms	Event
Cry-1Ac	1996	*Helicoverpa*, *Pectinophora*, *Earias* spp.	Bollgard® Delta and pineland/ Monsanto
Cry1Ac + Cry2Ab	2003	*Helicoverpa*, *Pectinophora*, *Earias*, *Spodoptera* spp.	Bollgard-II® Delta and pineland/Monsanto
Cry1Ac + Cry1Ab and or CpTi	1997	*Pectinophora*, *Earias* spp.	Bollgard-II® Delta and pineland/Monsanto
Cry1Ac + Cry1Fa	2005	*Helicoverpa*, *Pectinophora*, *Earias*, *Spodoptera* spp.	Wide Strike® Dow Agro Sciences
Vip3A	2005	*Helicoverpa*, *Pectinophora*, *Earias*, *Spodoptera*, *Agrotis* spp.	Vip Cot® Syngenta

Modified from Tabashnik et al. [12].

Table 1.
Generations of Bt and their potential benefits.

terpenoids and condensed tannins both alter the efficacy of Cry1Ac protein. Hydrolysable tannins combined with Bt toxins increased the mortality of lepidopterous larvae [17, 18]. Environmental stresses also can alter the efficacy of Bt protein in the field.

1.2 Factors affecting efficiency of Bt technology

Target larvae of lepidopterous species uptake different amount of Bt toxins by feeding on Bt-expressing parts of the plant [19]. Obrist et al. [20] also found that thrips, *Frankliniella tenuicornis* acquired Cry1Ab after feeding on Bt plants. Mellet and Schoeman [21] quantified Bt concentration in the body of insects with the help of enzyme linked immunological sorbent assay (ELISA) method. Sucking arthropods extract phloem but uptake very minute quantity of Bt toxin as compared with lepidopterous larvae [19, 22].

1.3 Bio-safety detection of transgenic crops

Pest species is suppressed by naturally occurring biological and environmental agents. Living organisms are found to kill, weaken or reduce the reproductive potential of pest species. Effectiveness of biological control agents is enhanced by "conservation" of biocontrol agent, i.e., to protect and maintain population of naturally existing biocontrol agents. Conservation techniques include reducing or eliminating insecticide applications. If the number of biocontrol agents are less than the pest species population existing in a locality, the biocontrol agents can be reared in laboratory and released to increase their population in that area. This is called as "augmentation of biocontrol agents." Sometimes few number of beneficial insects are needed to be released in several locations to suppress population of local insect pest, i.e., called inoculative release and in other case large numbers are released on a single locality, i.e., called as inundative release. If the population of biocontrol agents exists high in one locality they can also be redistributed to the new locality where there is pest problem and beneficial fauna is low. The biocontrol agents can also be imported from one country to the country where there is pest problem. All these techniques are very useful from pest management point of view and have very useful results as biological control against invertebrate pest species. Effectiveness of biocontrol agents is affected by environmental resistance/climatic adaptability, synchrony with life cycle of insect pest, insecticidal and Bt toxin effects.

Benefits and risks of transgenic crops are common concerns of Bt technology. Potential benefits are reduction in use of broad-spectrum insecticides. It produced an opportunity for conservation of biological control agents. It has less hazardous effects on non-target arthropods than insecticides. Its long term cultivation also had no significant impact on soil health [23]. However, effect of Bt insecticidal proteins on beneficial insects is given in **Table 2**

1.4 Risks of Bt on target and non-target species

However, there are also some putative risks related with development of Bt resistance in target insect pest [24] and direct or indirect effect on non-target arthropod biodiversity [25]. Pollen drift by out crossing in open pollinated crops and release of Bt toxin into soil through root exudation, natural wounding or root cell senescence and its effect on soil health as well as its degradation are the other risks of Bt technology [26].

1.5 Effect of Bt on predators

Little change in pest community has ability to change the community of associated beneficial fauna of natural enemies. It is due to direct feeding on transgenic plant tissue and indirectly due to change in food quality by emission of volatile exudates that can attract or repel the pest/non targeted arthropod fauna and ultimately affect the food-web [27]. Cry1Ac toxin of Bt plant is transferred through non-target herbivores to natural enemies at different trophic levels. Therefore, harmful effects on natural enemy population are appeared [28]. It is due to change in food-web [27]. Predators population is reduced either due to change in tritrophic interaction, i.e., shortage of pray or ill effect of Cry protein on beneficial [29].

1.5.1 Heteropteran predators

Geocoris punctipes and *Orius insidiosus* species are common and important members of the natural enemies of a variety of food crops worldwide. They are omnivorous feeders and their generalized feeders of sucking insect pest and acquire Cry1Ab from the body of insect herbivory [21, 30]. However, uptake of Cry1Ac toxin differed with respect to size and feeding requirement of the predators. It was comparatively high in spined soldier bugs, *Podisus maculiventris* that consumed considerably more prey biomass compared to small predatory heteropterans (*G. punctipes* and *O. insidiosus*) [31, 32]. Insecticidal Bt protein exerted adverse

Crop	Bt protein	Targeted arthropod	Tritrophic effect on Non-targeted arthropod	Detrimental effect	References
Cabbage	Cry1Ac	*Plutella xylostella*	Parasitoid	Not detected	[33]
Cabbage	Cry1Ac	*P. xylostella*	*Chrysoperla carnea* larvae	Detected	[33]
Rice	Cry1Ac, Cry2Aa or Cry1Ca	Brown Planthopper	Pond wolf spider	No	[34]
Cotton	Cry1Ac	*Helicoverpa armigera*	*C. carnea* larvae	Not detected	[31, 32]

Table 2.
Tritrophic impact of Bt on populations of the major beneficial predators.

effect on the biology of beneficial fauna. When heteropteran predators fed on *Spodoptera exigua* collected from Cry1Ac cotton field, effect of Bt on biological activity of predators were observed and longevity of *Orius tristicolor* and *Geocoris punctipes* was reduced as compared to non-transgenic treatments.

1.5.2 Effect of Bt on lady bug

Coccinellid beetles belong to very important and most diverse group of insects that have very useful predatory potential. Birch et al. [35] reported that when lady bugs consumed aphids that had picked Bt for 2–3 weeks, fecundity, egg viability and longevity ladybird longevity was reduced up to 51%. Adverse effects on ladybird reproduction, caused by eating peach-potato aphids from transgenic potatoes, were reversed after switching ladybirds to feeding on pea aphids from non-transgenic bean plants. These results demonstrated that expression of a lectin gene for insect resistance in a transgenic potato line can cause adverse effects to a predatory ladybird via aphids in its food chain. Zhao et al. [36] reported added Bt toxin Cry1Ah and Cry2Ab toxin to artificial diet of *Aphis gossypii* and same aphids were used as feed of coccinellid beetle to investigate the tritrophic effect of Bt on beetle. They reported that development, survival and pupae formation of predatory beetle was not affected by Bt crystalline protein.

1.5.3 Effect of Cry1Ac in spider

Cry1Ac toxin uptake was comparatively high in spined soldier bugs, *Podisus maculiventris* that consumed considerably more prey biomass compared to small predatory heteropterans (*G. punctipes*, *N. roseipennis* and *O. insidiosus*) [31, 32]. According to [34] transgenic Bt rice lines producing Cry1Ac, Cry2Aa or Cry1Ca had no detrimental effects on pond wolf spider (PWS), *Pardosa pseudoannulata*. However, Zhou et al. [37] reported that the activities of three key metabolic enzymes were significantly influenced in spider after feeding on fruit flies containing Cry1Ab.

1.5.4 Effect of BT on Chrysoperla

Larval stage of *C. carnea* has very useful predatory potential and rank an important status from pest management point of view. Mellet and Schoeman [21] reported 55.6% survivorship of *C. carnea* larvae after feeding *Spodoptera littoralis* reared on Bt-free isoline. However, the survivorship of *C. carnea* larvae fed *S. littoralis* reared on Bt was significantly reduced to 17.7%, indicating that there may be an additional negative effect of consuming the intoxicated prey source. Similarly Feeding effect of Bt prolonged development of *C. carnea* and increased larval mortality as reported by Romeis et al. [38]. However, Tian et al. [39] reported that Bt crystalline protein had no direct and indirect effect on *Chrysoperla*.

1.5.5 Effect of BT on rove beetle

Riddick and Barbosa [40] monitored numbers of *Lebia grandis*, a predatory ground beetle that specializes on Colorado potato beetle. The numbers of *L. grandis* were significantly smaller in transgenic and mixed fields. Garcia-Alonso et al., [41] reported no effects of exposure to the toxin Cry1Ab through Bt maize fed-prey on the performance and digestive physiology of the predatory rove beetle *Atheta coriaria*.

1.5.6 Effect of BT on pollinator

Honey bees are important cotton pollinators that visit the flowers of the same plant or randomly the flowers of several other plants and one-third of the crops are insect-pollinated. Non targeted insects are directly and indirectly affected by the toxin produced by the transgenic plants [42]. Likewise, honey bees are exposed to direct exposure of Bt during nectar collection and ingestion of contaminated pollen [43].

1.6 Effect of Bt on parasitoids

Parasitoids population is reduced either due to shortage of pray or ill effect of Cry protein [29]. Cui and Xia [44] also demonstrated that parasitoid population was highly reduced in the Bt cotton plots. Host larvae emerged from Bt protein had prolonged larval duration but with less weight of larvae, pupae and adult similarly emergence of parasitoids and adult longevity were also negatively affected on such diet [45]. Baur and Boethel [46] reported that development of *Cotesia marginiventris* inside the body of *Pseudoplusia includens* larvae fed on Bt cotton had reduced longevity and oviposition. Sometimes it becomes difficult for parasitoids larvae to complete development either due to the premature death of larvae or behavior of prey (Schuler et al., 1999); [47–49]. Baur and Boethel [46] reported that *Cotesia marginiventris* developing inside *Pseudoplusia includens* larvae after feeding on Bt cotton had reduced longevity and females with less number of eggs. Parasitoid larvae also could not complete their development because Bt-susceptible hosts were not able to survive on Bt leaves [48, 49].

1.7 Risks of Bt resistance in herbivorous arthropods

Bt technology has altered the arthropod community by reducing population of targeted lepidopterous larvae. This disturbance is mainly due to unavailability of prey for the predator/parasitoids. Mellet and Schoeman [21] reported highest mortality and delay in development of lepidopterous larvae after feeding on Bt as compared with control non-Bt plants. For clarity of results researchers have suggested that the effect of transgenic crops on arthropod community should be monitored on long term basis by using Simpson waiver index (SWI). This index is used to find out species richness [2].

This wide spread adoption of Bt by growers have also resulted in development of resistance in targeted arthropods to Bt crops and Bt sprays in the field [50]. Now many targeted lepidopteron species have field evolved resistance problem [51, 52]. *Plutella xylostella*, *Spodoptera exigua*, *Helicoverpa armigera* and *Pectinophora gossypiella* are the most common ones with field evolved resistance to Bt [53–56].

Bt-susceptible larvae of *Spodoptera exigua* from commercial non Bt-cotton fields contained around 2.7-fold less toxin in their bodies than resistant larvae from Bt. Similarly, *Tetranychus urticae* contained approximately 10-fold more toxin after feeding on Bt [57] (**Table 3**).

1.8 Use of eco-friendly, integrated pest management techniques

The suppression of pest population by the use of all suitable ways either through prevention, observation and intervention of arthropod pest species. Prevention means to manage pests rather than to eliminate them. In this case initial severity of pest is reduced through crop rotation, change in cropping pattern, plant breeding, changing of planting and harvesting time, use of trap crops as well as clean cultivation. Observation is related with pest scouting, harmful and beneficial insect

USA	Bollgard 1	2000	[58]
India	Bollgard 1	2003	[59]
China	Bollgard 1	1997	[53–56, 60]
Australia	Bollgard 1	2004	[61]
Pakistan	Bollgard 1	2009	[62]

Table 3.
Reports of Cry1Ac resistance in the world.

identification, decision of ETL (economic threshold level) and to determine when and what actions should be taken. Intervention is adaptation of various methods used to reduce the effect of economically damaging pest population. They all are the basic components of IPM (integrated pest management) including use of biological control, physical, cultural and chemical control.

1.9 Cultural control

It is the deliberate manipulation of environment to make it less suitable for the pest by eliminating its food, shelter and create hindrance to multiply insect pest on economic crops. The detail of cultural practices is given below.

1.9.1 Crop rotation

Rotations of crops and clean cultivation are the best cultural management tactics which can be used for the management of insect pest species. Sometimes movement of infested plants from one area serves as source of carryover to a new locality [63]. Preventive action that creates unfavorable condition for the pest will help to overcome such problem. More difficult is to decide cropping pattern year after year. Growing of same crop each year increases chance of pest problem. Crop cultivation serve as food chain of the arthropod herbivory and both arthropod population is interconnected with crop cultivation and planting date of the crops. It has been reported that highest population of arthropod species were present in the continuous cropping fields as compared with fallow fields. Fallow lands were used to break the life cycle of arthropod herbivory due to food shortage and un-availability of host plant [64]. Available crop also affects the feeding behavior of targeted arthropods. Many number of arthropods are monophagous (feed on single crop) or oligophagous (having two or narrow range of host plants). They will be naturally died due to unavailability of food by using crop rotation technique.

1.9.2 Trap cropping

Trap cropping technique practiced before the beginning of modern synthetic insecticides, is making a revival comeback now a day in many countries of the world [65]. The unique feature of trap crop is that it provides more attraction as food source and oviposition site than to the original economic crop [66]. Trap crops have been tested against many arthropod herbivores. In a dual choice test of plants, aphids preferred to lay eggs on brassica rather than wheat indicating that brassica has potential to serve as a trap crop for the management of wheat aphid [45]. Similarly, collard crop served as highly preferred for oviposition of *P. xylostella* than cabbage and it laid 300 times more eggs than on cabbage [67]. Trap crops are therefore recommended as an important tool for the management of arthropod herbivory and to protect the economic crops.

1.9.3 Planting and harvesting time

Change in planting and harvesting time creates discontinuity in food supply for the insect pest species. This technique is called as "phenological asynchrony." Crops are matured before or after the onset of insect pest incidence. So they escape insect pest attack and farmers easily manage insect pests on economic crop. Similarly planting density and plant spacing can affect the pest population and searching behavior of insect pest for food and oviposition site.

1.9.4 Induced resistance

Plant nutrition can influence the feeding, longevity and fecundity of arthropod herbivory. Some macro and micronutrients enhance the resistance mechanism of the plants against insect pest species. They also help the help the plant to compensate damage caused by insect pest. Fertilizer and irrigation application also help the plant to overcome environmental and arthropod feeding damage stress.

1.9.5 Use of plant resistance

The useful technique for the control of insect pests is plant resistance, rather than Bt property resistant plants have many physical and chemical characteristics that are used to overcome insect pest problem. It has been reported that resistant plants/varieties are less infested by insect pest herbivory than susceptible ones. In this way population of insect pest are managed on resistant crops and it is due to the food preference property of the pest [68, 69]. The other biological parameters of the pest are also affected on resistant plants due to presence of primary and secondary metabolites [68]. In this way they provide natural control of the pest without use of pesticides. Resistant plants are safe for natural enemies so also conserve and promote beneficial fauna [70].

1.10 Monitoring of pest population

Forecasting, monitoring and pest scouting are considered as important part to devise an IPM strategy. These techniques are used to determine the population and stage of insect pest infestation as well as the population of biocontrol. These are very useful techniques to decide action plan on the basis of economic threshold level of the pest and existing population of the predators. Pest monitoring is done by using various traps like pheromone, light, colored, sticky, pitfall and suction traps [71]. All of the traps have their unique importance because pheromone traps based on sex attraction are sensitive and species specific, light trap attract general pest and non-target flying insects, colored and sticky traps attract insect pest toward and their specific color, pitfall traps are used to trap soil dwelling insects and suction traps are used to suck minute soft bodied insects [72, 73].

1.10.1 Pheromone traps

However, sex pheromone traps are considered as valuable tools from monitoring point of view [74]. Due to specificity and least hazardous for non-target species pheromone traps are considered the best as compared with chemical control and are acceptable among various alternatives control. The first pheromone trap as a mating disruptant was used by [75] from *Bombyx mori*. Sex pheromone against pink bollworm, *Pectinophora gossypiella* was developed during 1970s. Chemists have identified 30 compounds with properties of pheromones and now they are commercially available for more than 300 targeted arthropod herbivory [76]. They are packaged in slow-release dispensers used as lures in traps for mass trapping of sexually active adults. In this way they create mating disruption. Their efficiency pheromone traps depend upon the ratios of the active component, dose rates and dispensers applied. They are very valuable source of insect pest monitoring because more male moths can be captured in trap even when population is at initial stage [77, 78].

1.11 Refuge crop for pest

A genetic change in population occurs due to the mortality of susceptible arthropod herbivory induced by continuous exposure of Bt toxin. Some out of these organisms survive due to natural tolerance against Bt toxin. Breeding of tolerant insects with each other develop ability to survive and changed to become a whole resistant population. In order to tackle the Bt resistance problem, growing of at least 5% area of non-Bt refugia around Bt crop is recommended. Basically non-Bt refuge allows the targeted arthropods to survive and reproduce. So resistance alleles would be suppressed by susceptible alleles. The lepidopterous insects that express resistance and survived in Bt crop would have chance to mate with susceptible ones grown on non Bt refugia. Susceptible generation produced on non Bt crop will compete for food, shelter and mating with resistant strain of targeted arthropod herbivory. The final result will be reduction in multiplication rate of insect therefore chances for development of susceptible strain are increased after many generations. Seed companies are now using technique of non Bt seed mixing with the Bt crops to suppress problem of Bt resistance. Commercial packets of Bt crop seed also contain premixed non-Bt seeds.

1.12 Refugia for beneficial arthropods

Uncultivated land can sustain a diverse range of beneficial fauna has long been known. Refugia may be located within the cultivated crop and outside the crop. Within crop natural refugia may exist in the form of unsprayed crop area, protected parts of the plant and alternative host plants. Outside the crop field borders, live fences, mixed cropping, intercropping and strip planting have been used to provide refugia for natural fauna. Generalist predators and parasitoids move among these crops depending upon the availability of their host (prey). However, effective use of refugia normally requires crop-pest and natural interaction. Such type of interaction

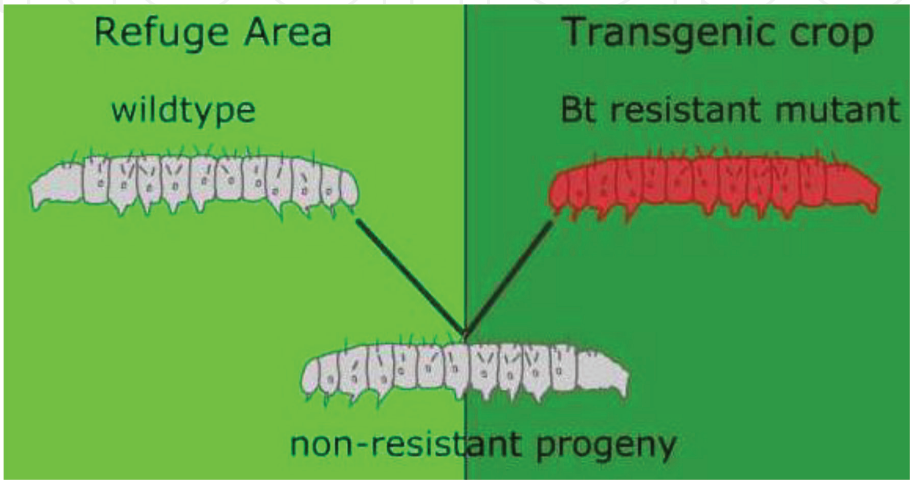

Figure 1.
Importance of non Bt refugia in reducing Bt resistance in targeted larvae.

is called as Tritrophic interaction. Effectiveness of interaction depends upon the time and space because floral composition of refugia, its location and area dimension as well as synchronization of pest and beneficial fauna play an important role from pest management point of view. Planting of *Medicago sativa* strips in cotton has been reported to increase the predatory fauna especially the beetles, *Chrysoperla carnea* that was 5–7 times more abundant on *Medicago* strips than on cotton. This beneficial fauna then migrated to cotton and its population was double on cotton with *Medicago* plots in comparison with cotton without. Refugia have dual function from pest management point of view it has ability to serve as trap crop for pest and conservation of natural enemies. It has also potential to serve as wind speed breaker and overwintering sites for parasitoids. Some natural enemies overwinter within the parts of the plants like wise predatory mite *Typhlodromus* spp., overwinter in calyx activities of apple fruit. The damaged fruits serve as refugia for it and should be left on ground during winter to enhance the population of this type of predatory mite (**Figure 1**).

2. Conclusion

Concerns of bio-safety has been and should be a compulsory component for the long lasting sustainability of transgenic/Bt technology. As mentioned earlier negative impact of Bt on some predators and parasitoids are actual therefore mode of action of Cry1Ac on predators should be investigated by testing of binding and pore formation capacity of toxins to epithelial vesicles of gut membrane, because activity of Cry1Ac toxin is specific for a particular predator. Further food preference difference of predator between Bt and non Bt crop should also be investigated. Difference between availability of semiochemicals in transgenic and conventional crops should also be evaluated because they have tendency to repel the beneficial arthropods. Population of predatory fauna is also badly affected in Bt plots due to reduction of food prey (arthropod herbivory). The key point of the review paper is that Bt crops are more dangerous for the parasitoids than predators. Therefore, we should focus our research on tritrophic impact of Bt crops on parasitoids. Moreover, it was concluded that refugia play an important role from pest management point of view; it can mitigate field evolved resistance in arthropod herbivory on one hand and can sustain the natural parasitoid/predatory fauna on the other hand.

3. Study Questions

1. What is Bt and its significance in pest management? Discuss in the light of modern outlook.

2. Discuss about different generations of Bt.

3. Enlist the factors affecting efficacy of Bt.

4. Elaborate about biosafety detection of transgenic crops for beneficial fauna.

5. Discuss about the Bt resistance problem in target insects.

6. Importance of Integrated Pest Management.

7. Useful of Non Bt Refugia.

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