

Impact of Different Transgenic Crops (*Bt*) on Insect Biocontrol Agents

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Abstract: The several transgenic crops viz., egg plant, broccoli, canola, chickpea, cotton, groundnut, maize/ corn, potato, tobacco and tomato, etc., have been reported as safe to the different insect biocontrol agents like predators and parasitoids. However, a few workers have shown the indirect effects of sorghum, soyabean, sugarcane and rice on these biocontrol agents. Over the chemical protection measures used for insect-pest control, use of transgenic crops is environmentally safe, effective and ecofriendly approach, which can be well suited in the IPM system. However, keeping in mind the several hue and cry issues regarding their health hazardous effects, there is a great need to extend such studies from lab to land with the more understanding of triangular interaction among pest, *Bt* and biocontrol agent.

Keywords: Impact, Transgenic crops, Insect biocontrol agents.

I. INTRODUCTION

Rachel Carson in *Silent Spring* (1962) supported the use of *Bacillus thuringiensis* (*Bt*) based products, a soil bacterium, which can kill various insects, as a biological control alternative to chemical pesticides. At the time *Silent Spring* was written, *Bt* was just beginning to be used in the United States. Spores and protein crystals of several *Bt* strains have been used as microbial insecticides since the 1950s [12]. Different δ -endotoxins e.g., the Cry1A and Cry1C were reported toxic to lepidopteran larvae of European corn borer (*Ostrinia nubilalis*), while Cry3A to coleopteran larvae like those of the Colorado potato beetle (*Leptinotarsa decemlineata*) [37]. However, in 1999 the total sales of *Bt* products constituted less than 2 % of the \$US 8 billion spent globally for all insecticides [67].

Transgenic tobacco crop [(expressing proteins of *Bacillus thuringiensis* (*Bt*)] was the first example for the control of insect-pests. Subsequently, transgenes were expressed in other crops, which benefitted the growers by conserving natural enemies, suppressing insect-pests & reducing insecticides. *Bt* brinjal with adverse effects on *Aphidius ervi*, *Encarsia formosa* and *T. chilonis*, respective parasitoids for aphid, whitefly, and shoot & fruit borer was reported [35]. *Bt* tomato caused no adverse effect on the biological parameters of generalist predator *Macrolophus caliginosus*, an endoparasitoid wasp, *Aphidius ervi* in the laboratory [45]. *Bt* cotton caused no harmful impact on chrysopids & coccinellids under field [14]. The *Bt* soyabean was reported as effective to reduce the target insect pests & favour the populations of natural enemies [76].

The published information available to date reveals no detrimental effects of *Bt*-TP's on the abundance or efficiency of biocontrol agents (BCA's). However, the indirect effects by few transgenic crops on the trophic occur, are considered due to local reductions of certain specialist parasitoids whose hosts are their primary targets. There is still an urgent need to have an improved understanding of pest-BCA-insecticide interactions which can help in shaping more effective pest management strategies.

II. COMMON PARASITIDS AND PREDATORS

Different insect predators and parasitoids are used as the BCA's against various insect-pests on various crops have been listed in tables 1 & 2 (Plates 1-37).

Table 1: Common egg/larval/pupal parasitoids used against different insect-pests on various crops

	Type of natural enemy	Pest (s)	Crop (s)
1.	Wasp, <i>Trichogramma japonicum</i> Ash. (Hymenoptera: Trichogrammatidae)	Top borer, <i>Scirpophaga excerptalis</i> Walker (Lepidoptera: Pyralidae)	Sugarcane
		Yellow stem borer, <i>Scirpophaga incertulas</i> Walker, plant hopper	Rice
2.	<i>T. chilonis</i> Ishii (Hymenoptera: Trichogrammatidae)	Leaf folder, <i>Cnaphalocrocis medinalis</i> Guenee (Lepidoptera: Pyralidae)	
		ABW, <i>Helicoverpa armigera</i> Hub. (Lepidoptera: Noctuidae)	Sunflower
3.	<i>T. pretiosum</i> Riley (Hymenoptera: Trichogrammatidae)	Cabbage looper, <i>Trichoplusia ni</i> Hub. (Lepidoptera: Noctuidae)	Brassicae
4.	<i>T. exiguum</i> Pinto & Platner (Hymenoptera: Trichogrammatidae)	Tobacco budworm, <i>Heliothis virescens</i> Fab. (Lepidoptera: Noctuidae)	Cotton
5.	<i>T. brassicae</i> Bezd. (Hymenoptera: Trichogrammatidae)	Leaf miner, <i>Tuta absoluta</i> Meyrick (Lepidoptera: Gelechiidae)	Tomato
		<i>H. armigera</i>	Maize
6.	Aphid parasitoid, <i>Aphidius rhopalosiphii</i> (Hemiptera: Aphididae)	Aphid, <i>Rhopalosiphum padi</i> (Hemiptera: Aphididae)	
		Rose-grain aphid, <i>Metopolophium dirhodum</i> (Homoptera: Aphididae)	Wheat
7.	Wasp, <i>Hyposoter didymator</i> Thunb. (Hymenoptera: Ichneumonidae)	Egyptian cottonworm, <i>Spodoptera littoralis</i> Boisid. (Lepidoptera: Noctuidae)	Cotton
8.	Aphid parasitoid, <i>Diaertiella rapae</i> M'Intosh (Hymenoptera: Braconidae)	Cabbage aphid, <i>Brevicoryne brassicae</i> L. (Homoptera: Aphididae)	
9.	Parasitic wasp, <i>Aphidius ervi</i> Haliday (Hymenoptera: Braconidae)	Potato aphid, <i>Macrosiphum euphorbiae</i> Thomas (Homoptera: Aphididae)	Tomato
10.	Encyrtid parasite, <i>Leptomastix dactylopi</i> Howard (Hymenoptera: Encyrtidae)	Mealy bug, <i>Planococcus citri</i> Risso (Hemiptera: Pseudococcidae)	
11.	Red scale parasite, <i>Aphytis melinus</i> DeBach (Hymenoptera: Aphelinidae)	California red scale, <i>Aonidiella aurantii</i> Maskell (Diptera: Cecidomidae)	Citrus
12.	Aphid parasite, <i>Aphelinus gossypii</i> Timb. (Hymenoptera: Aphelinidae)	Green aphid, <i>Aphis spiraeicola</i> (Homoptera: Aphididae)	
13.	Whitefly parasite, <i>Encarsia formosa</i> Gahan (Hymenoptera: Aphelinidae)	Greenhouse whitefly, <i>Trialeurodes vaporariorum</i> Westwood (Hemiptera: Aleyrodidae)	GH vegetables
14.	Solitary endo-larval parasitoid <i>Cotesia plutellae</i> Kurdjumov (Hymenoptera: Braconidae)		
15.	<i>C. flavipes</i> (Hymenoptera: Braconidae)	Diamond backmoth, <i>Plutella xylostella</i> Linn.	Crucifers
16.	<i>Diadegma insulare</i> Cresson (Hymenoptera: Ichneumonidae)		
17.	Ichneumonid wasp, <i>Campoletis chloridae</i> Uchida (Hymenoptera: Ichneumonidae)	<i>H. armigera</i>	Chickpea
		Aphids (Homoptera: Aphididae)	Groundnut
18.	<i>C. sonorensis</i> Cameron (Hymenoptera: Ichneumonidae)	Tobacco budworm, <i>Heliothis virescens</i> Fab. (Lepidoptera: Noctuidae)	Tobacco
19.	<i>Bracon hebetor</i> Say (Braconidae: Hymenoptera)	Lepidopteran larvae (Lepidoptera Noctuidae)	-
20.	Wasp, <i>Chelonus blackburni</i> Cam. (Hymenoptera: Braconidae)	Pink bollworm, <i>Pectinophora gossypiella</i> Saunders (Lepidoptera: Gelechiidae)	Cotton
21.	<i>Diadegma semiclausum</i> Hellen (Hymenoptera: Ichneumonidae)		
22.	<i>Oomyzus sokolowskii</i> Kurdj. (Hymenoptera: Eulophidae)	<i>P. xylostella</i>	Brassica

Table 2: Common predators used against different insect-pests on various crops.

Plate No.	Type of natural enemy	Pest (s)	Crop (s)
23.	Australian lady bird beetle, <i>Cryptolaemus montrouzieri</i> Mul. (Coccinellide: Coleoptera)	Grapewine mealy bug, <i>Maconellicoccus hirsutus</i> Green (Homoptera: Pseudococcidae)	Grape, guava, mango
24.	Convergent lady beetle, <i>Hippodamia convergens</i> Bugwoodwiki (Coleoptera: Coccinellidae)	<i>P. citri</i>	Citrus
25.	Eleven-spot ladybird, <i>Coccinella undecimpunctata</i> L. (Coleoptera: Coccinellidae)	Aphids (Homoptera: Aphididae)	Apple, pear, peach
26.	Spotted lady beetle, <i>Coleomegilla maculata</i> DgGeer (Coleoptera: Coccinellidae)	<i>Spodoptera frugiperda</i> Smith (Lepidoptera: Noctuidae,) & <i>Schizaphis graminum</i> Rondani (Hemiptera: Aphididae)	Sweet corn
27.	Asian lady bird beetle, <i>Harmonia axyridis</i> Pallas (Coleoptera: Coccinellidae)		
28.	Minute pirate bug, <i>Orius insidiosus</i> Say (Hemiptera: Anthocoridae)	Western flower thrips, <i>Frankliniella occidentalis</i> , Onion thrips,) <i>Thrips tabaci</i> , phytophagous mites	Several crops
29.	<i>Orius strigicollis</i> Poppius (Hemiptera: Anthocoridae)	Predaceous flower bug on <i>Thrips palmi</i> (Thysanoptera: Thripidae)	Flowers
30.	Green lacewing, <i>Chrysoperla carnea</i> Step. (Neuroptera: Chrysopidae)	European corn borer, <i>Ostrinia nubilalis</i> Hub. (Lepidoptera: Crambidae)	Maize
		Lepidopteran pests	Cotton Rice
31.	Generalist predator, <i>Macrolophus caliginosus</i> Wagne (Miridae)	Non-target aphid, <i>Macrosiphum euphorbiae</i> (Homoptera: Aphididae)	Tomato
32.	Two-spotted ladybird, <i>Adalia bipunctata</i> (Coleoptera: Coccinellidae)	Peach-potato aphid, <i>Myzus persicae</i>	Potato
33.	Chilean mite, <i>Phytoseiulus persimilis</i>	Two-spotted mite, <i>Tetranychus urticae</i>	Vegetables
34.	Predaceous mite, <i>Neoseiulus (=Amblyseius) fallacies</i> G.	Spider mites, <i>Tetranychus</i> spp.	GH vegetables
35.	Predaceous mite, <i>Typhlodromus occidentalis</i> Nesb.	<i>T. urticae</i>	
36.	Carabid beetles (Coleoptera)	Sucking pests	Several crops
37.	Striped earwig, <i>Labidura riparia</i> Pallas (Dermaptera: Labiduridae).	Eggs & larvae of several pests	Several crops

III. TRANSGENIC CROPS AND BCA'S

Expression of *Bt* gene in tobacco and tomato provided the first example of genetically engineered plants for insect resistance [4,74]. Subsequently, several *Bt* genes have been expressed in transgenic plants, including tobacco, potato, tomato, cotton, brinjal, rice, etc. Field performance of transgenic tomato plants for the first time for expressing δ -endotoxin gene was reported [13]. Though Cry1Ab protein was effective against tobacco hornworm, higher level of gene expression was needed for the control of tomato fruit worm (*Helicoverpa* sp). Results of field trials of *Bt* transgenic tobacco [27] and cotton [77] expressing truncated δ -endotoxin genes were encouraging.

In general Cry1, Cry2, 13 and Cry9 proteins affect Lepidoptera, Cry3, Cry7, and Cry8 proteins are active against Coleoptera, and Cry4, Cry10, and Cry11 proteins affect Diptera. Transgenic crops expressing the insecticidal proteins of *Bacillus thuringiensis* (*Bt*) have been commercially available in the U.S. since 1996 and their adoption continues to expand rapidly in the U.S. and other parts of the developed and developing world [30]. Up to now, *Bt* genes have been transferred to a large number of plant species, such as cotton, potato, rice, eggplant, oilseed rape, chickpea and so on (table 3).

Table 3: Different transgenic crops with *Bt* genes resistant against various insect-pests.

Sr. No.	Transgenic crop	Transgene (s)	Target insect-pest (s)
1.	Alfalfa	<i>Cry1C</i>	<i>Spodoptera littoralis</i>
2.	Egg plant	<i>Cry3b</i> <i>Cry1Ac</i>	<i>Leptinotarsa decemlineata</i> <i>Leucinoides orbonalis</i>
3.	Brassica	<i>Cry1C</i>	<i>Plutella xylostella</i> , <i>Trichoplusia ni</i> , <i>Pieris rapae</i>
4.	Canola	<i>Cry1Ac</i>	<i>Helicoverpa zea</i> , <i>S. exigua</i>
5.	Chickpea	<i>Cry1Ac</i>	<i>H. armigera</i>
6.	Cotton	<i>Cry1Ab</i> , <i>Cry1Ac</i> , <i>Cry2Ab</i>	<i>H. armigera</i> , <i>H. zea</i> , <i>Heliothis virescens</i> , <i>Pectinophora gossypiella</i> , <i>S. exigua</i> , <i>T.ni</i>
7.	Groundnut	<i>Cry1Ac</i>	<i>Elasmopalpus lignosellus</i>
8.	Maize /Sweet corn	<i>Cry1Ab</i> , <i>Cry1Ac</i> , <i>Cry9c</i>	<i>Ostrinia nubilalis</i> , <i>Chilo partellus</i> , <i>Busseola fusca</i>
9.	Poplar	<i>Cry1Aa</i>	<i>Lymantria dispar</i>
10.	Potato	<i>Cry1Ab</i> , <i>Cry1Ab6</i> <i>Cry3A</i> , <i>Cry3B</i> <i>Cry1Ac9</i> , <i>Cry5-Bt</i>	<i>Phthorimaea operculella</i> <i>L. decemlineata</i> <i>P. operculella</i>
11.	Rice	<i>Cry1Ab</i> , <i>Cry1Ac</i> , <i>Cry2Aa</i>	<i>C. suppressalis</i> , <i>Cnaphalocrocis medinalis</i> , <i>Scirpophaga incertulas</i>
12.	Sorghum	<i>Cry1Ac</i>	<i>C. partellus</i>
13.	Soyabean	<i>Cry1Ac</i>	<i>H. virescens</i> , <i>H. zea</i>
14.	Sugarcane	<i>Cry1Ab</i>	<i>Diatraea saccharalis</i>
15.	Tobacco	<i>Cry1Ab</i> , <i>Cry1Ac</i> , <i>Cry2a5</i>	<i>H. virescens</i> , <i>M. sexta</i> , <i>H. armigera</i>
16.	Tomato	<i>Cry1Ac</i>	<i>M. sexta</i>

IV. GLOBAL AREA UNDER TRANSGENIC CROPS

The *Bt* crops were grown on nearly 170 million ha in 28 countries in 2012 [29] (table 4). *Bt* crops have provided economic benefits to growers and reduced the use of other insecticides [43, 53,67], suppressed pest populations on a regional basis [9,28], conserved natural enemies [70] and promoted biological control services in agricultural landscapes [43].

All currently available insect resistant GM plants are resistant to a limited number of herbivorous insects because of the high degree of pest specificity of *Bt*.

Table 4: Global Area of Biotech Crops in 2012: by Country (Million hectares).

Rank	Country	Area (mha)	Biotech crops
1.	USA	69.5	Maize, soybean, cotton, sugarbeet, alfalfa, papaya, squash
2.	Brazil	36.6	Soybean, maize, cotton
3.	Argentina	23.9	Soybean, maize, cotton
4.	Canada	11.6	Canola, maize, soybean, sugarbeet
5.	India	10.8	Cotton
6.	China	4.0	Cotton, papaya, poplar, tomato, sweet pepper
7.	Paraguay	3.4	Soybean, maize, cotton
8.	South Africa	2.9	Maize, soybean, cotton
9.	Pakistan	2.8	Cotton
10.	Uruguay	1.4	Soybean, maize
11.	Bolivia	1.0	Soybean
12.	Philippines	0.8	Maize

13.	Australia	0.7	Cotton, canola
14.	Burkina Faso	0.3	Cotton
15.	Myanmar	0.3	Cotton
16.	Mexico	0.2	Cotton, soybean
17.	Spain	0.1	Maize
18.	Chile	<0.1	Maize, soybean, canola
19.	Colombia	<0.1	Cotton
20.	Honduras	<0.1	Maize
21.	Sudan	<0.1	Cotton
22.	Portugal	<0.1	Maize
23.	Czech Republic	<0.1	Maize
24.	Cuba	<0.1	Maize
25.	Egypt	<0.1	Maize
26.	Costa Rice	<0.1	Cotton, soybean
27.	Romania	<0.1	Maize
28.	Slovakia	<0.1	Maize

V. WAYS OF EXPOSURE OF BCA'S TO *Bt* PROTEINS

- (i). Direct exposure as a result of herbivory, e.g. when a natural enemy feeds on pollen or plant sap from a transgenic plant.
- (ii). Exposure through honeydew, e.g. in contrast to current *Bt* crops, certain experimental lectin or protease inhibitor expressing plants are known to transport insecticidal proteins in the phloem. When sap sucking insects, such as aphids, feed on such plants, the insecticidal proteins are likely to appear in their honeydew [36,54,68]
- (iii). Indirect exposure when a natural enemy feeds or parasitizes a target herbivore containing the transgenic product, e.g. when a natural enemy feeds or parasitizes a *Bt*-fed caterpillar.
- (iv). Indirect exposure when a natural enemy feeds or parasitizes a non-target herbivore containing the transgenic product, e.g. when a natural enemy feeds or parasitizes a *Bt*-fed thrips [61].

VI. DIFFERENT TRANSGENIC CROPS

1. Alfalfa

GM alfalfa is resistant to Monsanto's glyphosphate herbicide, commercially known as Roundup. This decision to completely deregulate the crop (except to see large acreages planted this spring) surprised some observers, since the agency's own recently-completed environmental impact statement (EIS) suggested two additional alternatives involving some level of regulation for the crop, such as not allowing it to be grown where it is likely to contaminate conventionally or originally grown alfalfa.

2. Eggplant

Parasitoids:

No adverse effects were found for parasitoid, *Aphidius ervi* developing on aphid hosts feeding on *Bt* eggplants as compared to the isogenic control line. Mortality and longevity of parasitoids from *Bt* eggplants (Cry3B) was not different when compared to parasitoids from isogenic eggplants. Different physiological traits during the growing stages of these plant varieties had an indirect effect on the herbivore-parasitoid-system. *Bt* eggplants do not cause any adverse effects on *Encarsia formosa* when parasitizing the whitefly nymphs as compared to isogenic eggplants. The *Bt* eggplants had not an influence on the development of the *Encarsia* [73]. The *Btk* (92.15% adult emergence) was reported as highly safe to the *T. chilonis*, a endoparasitoids of brinjal fruit and shoot borer [35].

3. Brassica

Parasitoids/predators:

Four *B. thuringiensis* (*Bt*) formulations, CM-134 and pirimicarb were found non toxic to *C. plutellae* adults, endoparasite on *P. xylostella* in cruciferous vegetables [1]. The toxicity of *Bt* (0.1%) to the pupae of *D. semiclausum*, natural enemy of diamond back moth in cruciferous crops proved them to be relatively less toxic than to the adults because of protective barrier [72]. When *P. xylostella* (resistant to Cry1C) was allowed to feed on *Bt* plants and then become parasitized by *Diadegma insulare*, an important endoparasitoid of *P. xylostella*. The results indicated that the parasitoid was exposed to a biologically active form of the Cry1C protein while in the host but was not harmed by such exposure [10]. Studies were carried out over multiple generations by using *Bt* broccoli (expressing Cry1Ac), *Plutella xylostella* populations with a low frequency of individuals resistant to Cry1Ac and spinosad, and a predator, *Coleomegilla maculata*. The results revealed low populations of *P. xylostella* after 6 generations in treatment containing *C. maculata* and unsprayed *Bt* plants with non-*Bt* refuge showed slower resistance to *Bt* plants. In contrast, *Bt* plants without refuge were completely defoliated in treatments without *C. maculata* after 4-5 generations. In treatment with sprayed non-*Bt* refuge plants and *C. maculata*, the *P. xylostella* population was low, although the speed of resistance selection to Cry1Ac significantly increased. Thus, these studies concluded that natural enemies can delay resistance to *Bt* plants and have significant implications with *Bt* crops [40].

4. Canola

Parasitoids/predators:

Although *Bt* canola lines have not been commercialized in Europe yet, the impact on non-target organisms of these lines were investigated in several studies [20, 54, 60, 61, 71, 78]. Although aphid parasitoid, *Diaeretiella rapae* is a generalist parasitoid and is able to parasitize many aphid species, and is associated to cruciferous plants and parasitization rates are in general higher on hosts on these plants [51, 75]. The direct toxicity to *C. carnea* larvae was reported when *Bt* toxins were incorporated in oilseed rape [61]. Studies on adverse effects of *Bt* canola (Cry1Ac) on *D. rapae* of mealy cabbage aphid, *Brevicoryne brassicae*, revealed that the foraging efficiency and oviposition behaviour of this parasitoid species is not significantly influenced by *Bt* canola plants and expression of Cry1Ac had no detrimental effects on the development of the parasitoid larvae. Furthermore, the *Bt* canola variety did not impact the development of the parasitoids [73].

5. Chickpea:

Parasitoids:

Low levels of parasitization of *H. armigera* larvae by *Campoletis chloridae* on *Bt* chickpea (Cry1Ab or Cry1Ac) were recorded [59]. They revealed several life-table parameters of parasitoids to be negatively affected when the host larvae were exposed to *Bt* toxin (Cry1Ab or Cry1Ac) dissolved in artificial diet prior and/or after parasitization. However, ASAL (*Allium sativum* leaf lectin that targets aphid) @ 0.1% in the artificial diet, on which the *H. armigera* larvae were reared before and/or after parasitization, did not produce significant adverse effects on the survival and the development of the parasitoid. In general, there are no serious effects of *Bt* transgenic crops on the generalist predators. However, parasitoid activity is affected when their insect hosts are affected by the *Bt* toxins in transgenic plants [58].

Chickpea mediated effects of *Bt* on survival of *H. armigera* and its parasitoid, *C. chloridae*

Resistance or susceptibility of the chickpea genotypes to *H. armigera* did not affect cocoon formation of *C. chloridae*, indicating that Helicoverpa-resistant chickpea genotypes are compatible with *C. chloridae*. Adverse effects of *Bt* toxins on *C. chloridae* are mainly because of early mortality of *Bt*-fed *H. armigera* larvae, and slow growth and poor quality of the insect host [64]. The *Bt* chickpea results in prolonged the larval period of the parasitoid raised on *Bt* intoxicated larvae of *H. armigera* [65], and reduced cocoon formation (44.2 to 75.0%) and adult emergence of *C. chloridae* [15].

Carryover effects of *Bt* on *C. chloridae*

There were no significant carryover effects of *Bt* on survival and fecundity (Fig. 2) of *C. chloridae* in the F₂ generation. Cocoon formation was significantly greater on *H. armigera* larvae reared on ICCV 10 and C 235 than on ICC 506 and L 550, suggesting that chickpea genotypes have indirect effects on the parasitoid through poor survival and development of

H. armigera. They also studied the effects of *Bt* toxins on the *H. armigera* larval parasitoid, *C. chloridae* (Plate 4) through *Bt* intoxicated larvae on chickpea [15].

Detection of *Bt* in *H. armigera* and its parasitoid, *C. chloridae*

The ELISA test detected *Bt* proteins in the *H. armigera* larvae fed on *Bt* treated chickpea plants. No *Bt* proteins were detected in the larvae, cocoons and adults of *C. chloridae* reared on *Bt* intoxicated *H. armigera* larvae [15].

6. Cotton:

Parasitoids:

The *Bt* cotton was produced by transferring the cry (crystalline) gene from *Bacillus thuringiensis*, which is a soil bacterium that produces Cry insecticide proteins [80]. Minor effect of *Bt* cotton on natural enemy population in comparison with the alternative use of broad-spectrum insecticides was observed which reduced the natural enemies population up to 48 %. Most of *Bt* cotton that express Cry1 protecting plants from lepidopteran pest damages and have high level of resistance to primary pest (target pest) especially *H. armigera* [69]. The *Bt* cotton was grown in nine countries including USA, Argentina, Brazil, India, China, South Africa, Australia, Mexico and Colombia [31]. Several studies in recent years have examined the effect of *Bt* crops on natural enemies [8]. Based on data from 1990 to 2010 at 36 sites in six provinces of northern China, a marked increase in abundance of three types of generalist arthropod predators (ladybirds, lacewings and spiders) was there and a decreased abundance of aphid pests associated with widespread adoption of *Bt* cotton and reduced insecticide sprays in this crop was there. There was also found evidence that the predators might provide additional biocontrol services spilling over from *Bt* cotton fields onto neighbouring crops (maize, peanut and soybean) [43]. The *Bt* cotton was reported not to affect immature parasitoid, *E. formosa* mortality but it affected development time up to adult for *E. formosa*. Parasitoid reached the adult stage faster on non-*Bt* cotton over *Bt*. The transgenic cotton effects on parasitoid were complex but generally interpretable in terms of host whitefly quality variation among host plants used as food by the whiteflies during their development [3]. No significant influence of *Bt* cotton on abundance of natural enemies of crop pests viz., chrysopids, ladybird beetles was observed suggesting that there were no adverse effects of *Bt*-cotton on the natural enemies under field conditions [14]. Parasitization of mealy bug, *Phenacoccus solenopsis* Tinsley on *Bt* cotton by hymenopteran parasitoids viz., *Aenasius bambawaei*, *A. dactylopii*, *Hibiscus eytelweinii*, *Promuscidea pulchellus* and *P. unfasciiventris* ranged between 7.18 to 61.49% and 16.67 to 75.00%, respectively during year 2007-08 and 2008-09, with peak parasitization of 54.69 and 61.49%, respectively, during 44th and 1st meteorological week [5].

Predators:

Monitoring of *Bt* cotton has so far also failed to show any significant effects on predators, including *C. carnea* [77]. Several efforts have been made to determine the effect of *Bt* crops (produce *Bt* toxin) on non target organisms and some negative effects have been reported [24]. The negative side effects on *C. carnea* described in the laboratory [23] have so far not been reflected in terms of reduced populations in the field. The number of predators and parasitoids may decline owing to prey or host depletion in highly resistant TPs, but their persistence is not necessarily threatened if other nearby crops support acceptable host or prey species, or if their host range includes species other than the target pests [18]. Field experiments with *Bt* cotton [19] have so far shown little reduction in beneficial insect populations as a whole, but large-scale commercial planting of highly resistant plants is bound to have repercussions for species specific to target pests [18]. Combined effect of natural enemies and sublethal exposure to *Bt* cotton expressing Cry1Ac on the survival of bollworm larvae (*Helicoverpa zea*). Sublethal exposure was achieved by rearing larvae for 1-4 days on *Bt* cotton in the laboratory before transferring the survivors to untransformed cotton in the field. There was no difference in *H. zea* survival between transgenic and untransformed plants when natural enemies were excluded. However, when natural enemies were present, larvae exposed to sublethal doses of *Bt* cotton survived at lower rates than larvae reared entirely on untransformed cotton [46].

7. Groundnut:

Parasitoids:

No significant information on the influence of transgenic crop on natural enemies is available but in general, however, in a report, maximum parasitization of groundnut pests by parasitoid, *Campoletis chloridae* on *Bt* groundnut (Cry1Ac) was reported [59].

8. Maize/Corn:

The use of *Bt* maize has been associated with an 0.82 metric tonnes reduction in insecticide active ingredient and a 5.3% reduction in the environmental impact quotient (EIQ) [7]. Reductions in EIQ in other adopting counties have ranged from 60% in Canada, 33% in Spain, 26% in South Africa and 0% in Argentina. Estimated reductions in insecticide use in Argentina, South Africa, Spain and the USA are 0, 10, 63 and 8 %, respectively [53]. The highly publicized, preliminary laboratory assay was on the effect of *Bt* corn pollen on the monarch butterfly which revealed that larvae of monarch butterfly, *Danaus plexippus*, reared on milkweed leaves dusted with pollen from *Bt* corn, ate less, grew more slowly and suffered higher mortality than larvae reared on leaves dusted with non-*Bt* corn pollen or on leaves without pollen [42]. While this study has been strongly criticized for its poor methodology and interpretation [26,66], it continues to circulate. A series of papers published from laboratory and field experiments over a two-year period concluded that the effect of *Bt* corn pollen on monarchs under present field conditions is 'negligible' [62]. Moreover, nor does this work mentions a large number of follow-up studies, which the Pew Charitable Trust describes as showing the risk of GM corn to butterflies as "fairly small, primarily because the larvae are exposed only to low levels of the corn's pollen in the real-world conditions of the field."

Parasitoids:

Public concern about the potential negative impact of transgenic *Bt* maize varieties expressing Cry1Ab toxin on non-target organisms was fuelled by recent studies on predators and parasitoids reporting higher mortality of these organisms when fed with non-target herbivores which were reared on *Bt* plants or diet in the laboratory [11,21, 23,24]. Transgenic *Bt* sweet corn varieties were released on a limited basis in 1998 by Syngenta Crop Protection, Inc. (Greensboro, NC) (formerly Novartis Seeds, Inc.). These varieties express the Cry1Ab toxin from *Bt* (Berliner) [44]. While the control of sweet corn lepidoptera by *Bt* differs depending on whether it is applied foliarly or expressed by the plant, *Bt* has low toxicity to natural enemies, regardless of the delivery system [49]. The transgenic *Bt* sweet corn was reported as less toxic to the most abundant predators, *C. maculate*, *H. axyridis* and *O. insidiosus* [47]. Due to the absence of Cry1Ab toxins in aphid hosts, *Aphidius rhopalosiphii*, harm to parasitoid, *Rhopalosiphum padi* is unlikely to exist [55]. Adverse effects of *Bt* maize (Cry1Ab) on efficacy of *R. padi*, an aphid parasitoid of *A. rhopalosiphii*, and revealed no behavioural changes of female parasitoids. The females did not discriminate between *Bt* or non-*Bt* maize-herbivore insect systems. Parasitization of *R. padi* did not differ on *Bt*, isogenic or a conventional maize line. The study also concluded that *Bt* maize has no adverse effects on this aphid parasitoid with regard to their foraging efficiency and oviposition behaviour [73].

The *H. armigera* was sublethally affected when feeding on *Bt*-maize resulting in a mortality of 79 % to late instars. Mortality of larvae was dependent to a large portion on variation of *Bt* toxin expression and nutritional value of these plants. *Helicoverpa* eggs from a moth reared on *Bt* maize were of poor quality for the egg parasitoid *Trichogramma brassicae* resulting in a low performance of F1 females [73]. Clear evidence of such prey-quality mediated effects was reported [17], with a reduced *C. carnea* larvae survival when the predator was reared with *Bt* (Cry1Ab) maize-fed caterpillars (*S. littoralis*) but not when provided with *Bt* maize-fed spider mites (*T.urticae*), both containing biologically active *Bt* protein [48]. As caterpillars are affected by the *Bt* protein while spider mites are not, the authors concluded that *C. carnea* survival was compromised as a result of eating sublethally affected ("sick") prey, rather than by the *Bt* protein. Subsequent studies confirmed that Cry1A does not cause a direct effect on *C. carnea* larvae [38,56,59] and adults [39], contradicting earlier reports of direct toxicity [24].

Based on tritrophic studies on *Bt* maize and cotton, reduced developmental rates, reproduction and survival of parasitoids as a group were reported when exposed to *Bt* plants than those of high-quality hosts, where parasitoid development and survival were equivalent on hosts exposed or not exposed to *Bt* proteins. There was even a slight increase in reproductive performance when parasitoids were provided with high-quality hosts exposed to *Bt* proteins, compared with non-*Bt* controls. In case of predators, they revealed these to show slightly lower survivorship when provided compromised (low-quality) prey exposed to *Bt* proteins, but slightly faster developmental rates when provided unsusceptible (high-quality) prey exposed to *Bt* proteins. All other predator life history characteristics were unaffected by *Bt* proteins regardless of prey quality [70].

When *B. tabaci* exposed to one attack by *E. formosa*, had 90-100% parasitism upon dissection 5 days after parasitization, indicating high acceptability of 3rd instar *B. tabaci* from *Bt* and non-*Bt* cotton to *E. formosa*. In non-*Bt* cotton, *B. tabaci* mortality was 5 individuals out of 50 died during the studies. There was no significant difference in total parasitoid

mortality from oviposition to adult emergence between the *Bt* and non-*Bt* cotton. However, the wasps were smallest when emerging from whiteflies on the *Bt* cotton and largest on the non-*Bt* cotton [3].

The fecundity of female *E. formosa* provided with *B. tabaci* on cotton was strongly affected by cotton line. Parasitoid on non-*Bt* cotton significantly more whiteflies parasites. The fecundity of parasitoid was affected by the age and host plant interaction. Young parasitoid in both cotton lines parasitized more whiteflies [3].

Predators:

Feeding of transgenic maize pollen containing the lepidopteran-specific *Bt* toxin Cry1Ab to the ladybird *C. maculata*, the anthocorids bug, *O. insidiosus* and the lacewing *C. carnea*, caused no acute detrimental effects on pre-imaginal development and survival. They also did not observed detrimental effects on the abundance of coccinellid, anthocorid and chrysopid predators of *O. nubilalis* on transgenic *Bt* maize. After placing laboratory-reared European corn borer eggs in microplots in Michigan [52]. The *Bt* corn had no effect on numbers of adults and larvae of *C. maculata* or *O. insidiosus*, nor any effect on parasitism by *Macrocentrus grandii* Goidanich or *Eriborus terebrans* (Gravenhorst) [50].

No significant differences in egg predation, egg parasitism or predator densities between *Bt* (Cry1Ab) and non-*Bt* maize plots was observed. Predators of *O. nubilalis* monitored in this study included coccinellids (mainly *C. maculata*), lacewings and the anthocorid *O. insidiosus*. An additional objective of this field study was to monitor larval parasitism. As *O. nubilalis* larvae do not survive on transgenic *Bt* maize planted microplots of untransformed maize within the large plots of *Bt* maize and infested them artificially with *O. nubilalis* eggs. Overall parasitism levels of the resulting *O. nubilalis* larvae did not differ between the *Bt* and the untransformed plots, but only one species of parasitoid was found in the *Bt* plots, compared with the two species in the untransformed maize plots [50].

It was observed that feeding prey that had been reared on Cry1Ab maize to *C. carnea* increased larval mortality from 37% to 62%. In this study, two species of prey were tested: *O. nubilalis* (the primary target species of *Bt* maize) and the Egyptian leafworm, *Spodoptera littoralis* (a non-target species). In addition to a greater larval mortality, the development time of *C. carnea* larvae was prolonged when they were reared on *Bt*-fed *O. nubilalis* but there was no such effect with *S. littoralis* [23]. The *Bt* maize [50,52] have so far shown little reduction in beneficial insect populations as a whole, but large-scale commercial planting of highly resistant plants is bound to have repercussions for species specific to target pests [18]. An increased mortality and prolonged development was observed when *C. carnea* larvae were reared on pest caterpillars that had ingested corn leaves expressing Cry1Ab. Such experimental design did not permit a distinction between a direct effect of the *Bt* protein on the predator versus an indirect effect of consuming a sub-optimal diet consisting of sick or dying prey that had succumbed to the *Bt* protein; no firm conclusions could be reached [23]. Even ignoring the considerations of the validity of the laboratory methods, the authors state that no conclusions can be drawn because it was not known how results from laboratory trials might translate to the field [23] and that TPs are still more environmental friendly than most, if not all, chemical insecticides [23]. In addition, further work in which the predator was directly fed Cry1Ab confirmed that lacewings were not harmed by this protein [57].

9. Poplar

The perusal of literatures showed no work has been carried over regarding the impact of transgenic poplar on the natural enemies.

10. Potato

Predators:

Coccinellidae (ladybirds), appear to locate host habitats by random searching [25]. The studies on effect of transgenic *Bt* potatoes on the coccinellid, *Hippodamia convergens*, revealed the plants to express a coleopteran-specific *Bt* toxin and the prey consisted of peach-potato aphids (*Myzus persicae*), with no effects on survival, consumption of aphids, development or reproduction were observed in *H. convergens* [16]. The tachinid fly, *Myiopharus doryphorae* was able to develop in larvae of the Colorado potato beetle treated with sublethal *Bt* doses, but the emergence of healthy adults was reduced from 90% to 78% [41]. In tritrophic studies, 38% reduction in fecundity, 50% reduction of female longevity, lower egg hatch, no reduction in prey consumption of two-spotted ladybird, *Adalia bipunctata* were reported when they were fed on aphid, *M. persicae* on *Bt* potato (GNA lectin) over the non-*Bt* potatoes, concluding that the expression of a lectin gene for insect resistance in a *Bt* potato line can cause adverse effects to a predatory ladybird via aphids in its food chain [6]. No negative effects for *Geocoris punctipes* (Hemiptera: Lygaeidae) and *Nabis* spp. (Hemiptera: Nabidae) as

predators of *Epilachna varivestis* (Coleoptera: Coccinellidae) were reported when fed directly on *Bt* potato foliage [2]. Different prey species of a generalist predator had different effects on the development of resistance by Colorado potato beetle, *Leptinotarsa decemlineata* to *Bt* potato: the presence of one prey species delayed resistance while the other accelerated resistance [40].

11. Rice

Predators:

In studies on effect of Cry2Aa on *C. sinica* larvae in rice by using a non-target (*Laodelphax striatellus*) and a target herbivore (*C. suppressalis*) as prey and revealed that *C. sinica* larvae when fed with *L. striatellus* nymphs (reared on either *Bt* or control rice plants) are not sensitive to Cry2Aa at concentrations exceeding the levels that the larvae may encounter in *Bt* rice fields. However, detrimental effects in *Bt* rice-fed *C. suppressalis* as prey can be attributed to the decreased prey quality due to the sensitivity of *C. suppressalis* larvae to Cry2Aa [79].

12. Sorghum

The lack of requisite literatures regarding on the side effects of transgenic sorghum on the natural enemies shows no work done in this field. However, moderate levels of host parasitization by the parasitoids on *Bt* sorghum [59].

13. Soybean

Resistant soybean plants result into reduction in growth and fecundity in host and can lead to reduced larval survival, adult size, fecundity and reduced adult emergence of parasitoids [22]. On the basis of the occurrence and abundance of target and non-target pests in *Bt* and glyphosate-tolerant soybeans (RR1 and RR2), with and without the application of insecticides, *Bt* soybean was found effective to significantly reduce the target insect pests and favour the populations of natural enemies [76].

14. Sugarcane

Parasitoids:

In studies on *C. flavipes*, a parasitoid of the non-target pest, *Diatraea saccharalis* (Lepidoptera: Crambidae), small to marginal negative effects of artificial diet containing transgenic sugarcane tissue were observed on the rate of host suitability, number of cocoons and adult parasitoids emerging per host, per cent cocoons yielding parasitoids, and sex ratio and adult lifespan of parasitoids. These effects were variable between the two parasitoid generations examined. In contrast, differences were not detected between diet treatments in rates of host acceptance, egg load of females, and egg to adult developmental periods. The negative effects of transgenic sugarcane on *C. flavipes* detected in this study are important because GNA levels in the diet ($\approx 0.49\%$ of total protein content) containing transgenic sugarcane tissue were $\approx 50\%$ of the level expressed in transgenic sugarcane plants [63].

15. Tobacco

Parasitoids:

The *Bt* was first introduced into tobacco plants in 1987 [74]. Parasitism by younger larvae of tobacco budworm, *Heliothis virescens* by ichneumonid wasp, *Campoletis sonorensis* was reduced on *Bt* tobacco than on non-*Bt*, when pest larvae were exposed to parasitoids for 1-4 hours, which was supposed to be due to differences in host behaviour. By contrast, parasitism of older larvae exposed for several days on *Bt* tobacco plants was increased compared with that of larvae on control plants [33]. Prolonged development of first-instar *H. virescens* on *Bt* plants, leading to an extended 'window' for parasitism, was proposed as one likely mechanism for the synergism observed with *C. sonorensis*. A field trial with early *Bt* and CpTI tobacco lines found no effect of TPs on predatory bugs of the family Nabidae [27]. Field trials with low-expressing *Bt* tobacco lines indicated a synergistic interaction between the TPs and the parasitoid *C. sonorensis* in controlling *H. virescens* [33]. Several laboratory and glasshouse studies have revealed natural enemies to be adversely affected only when *Bt*-susceptible, sublethally damaged herbivores when were used as prey or host, with no indication of direct toxic effects, while field studies have confirmed that the abundance and activity of parasitoids and predators are similar in *Bt* and non-*Bt* crops. However, in contrast, they revealed conventional insecticides to impart negative impacts on natural enemies [34].

16. Tomato**Parasitoids/predators:**

Effects of *Bt*-tomato (Cry3Bb) on generalist predator *Macrolophus caliginosus* Wagner, an endoparasitoid wasp, *Aphidius ervi* Haliday and non-target aphid, *Macrosiphum euphorbiae* (Thomas) in laboratory conditions revealed no significant differences between performance of *M. euphorbiae* on *Bt* tomato (line UC82*Bt*) with respect to their near-isogenic control line (line UC82). Immunoassays did not detect Cry3Bb protein in *M. euphorbiae* developing on *Bt*-tomato. Similarly, no significant differences were found on the longevity and prey consumption of *M. caliginosus* when fed aphids reared on UC82*Bt* or on UC82. Moreover, the genetic modification did not affect the attractiveness of uninfested tomato plants toward *A. ervi*. They revealed that *Bt* tomato having no adverse effects on the biological parameters of *M. caliginosus*, *A. ervi* and *M. euphorbiae* [45].

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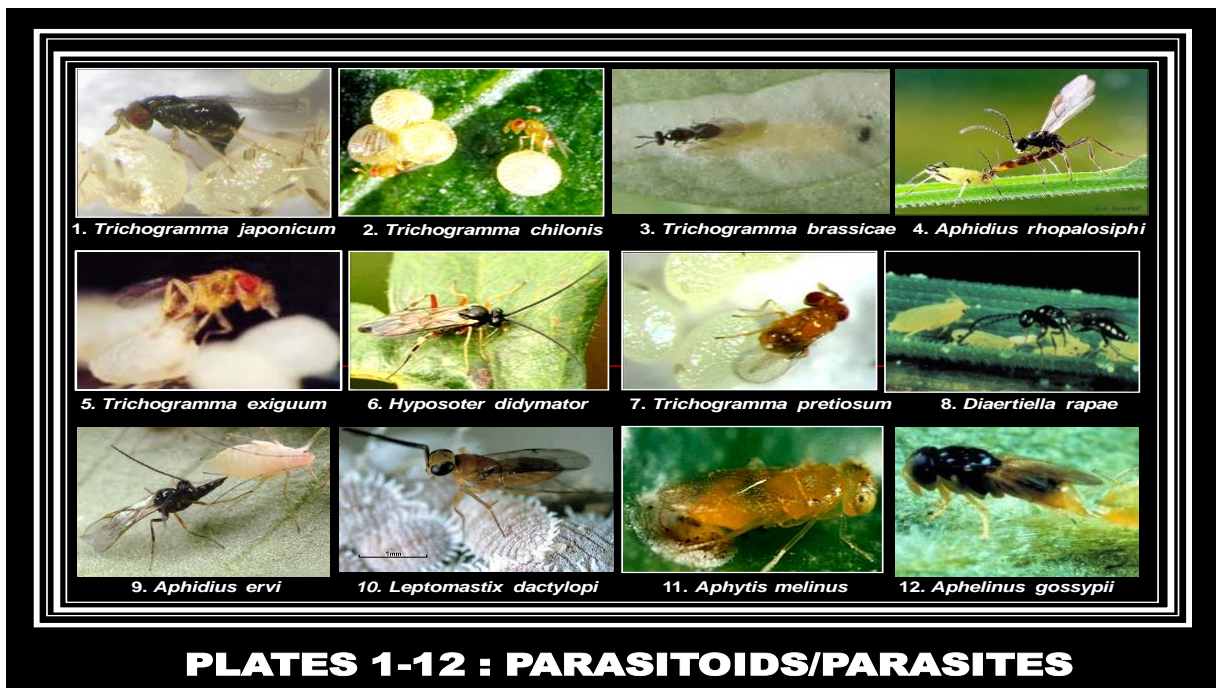
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APPENDIX - A

List of Figures:





23. *Cryptolaemus montrouzieri*

24. *Hippodamia convergens*

25. *Coccinella undecimpunctata*



26. *Coleomegilla maculata*



27. *Harmonia axyridis*



28. *Orius insidiosus*



29. *Orius strigicollis*

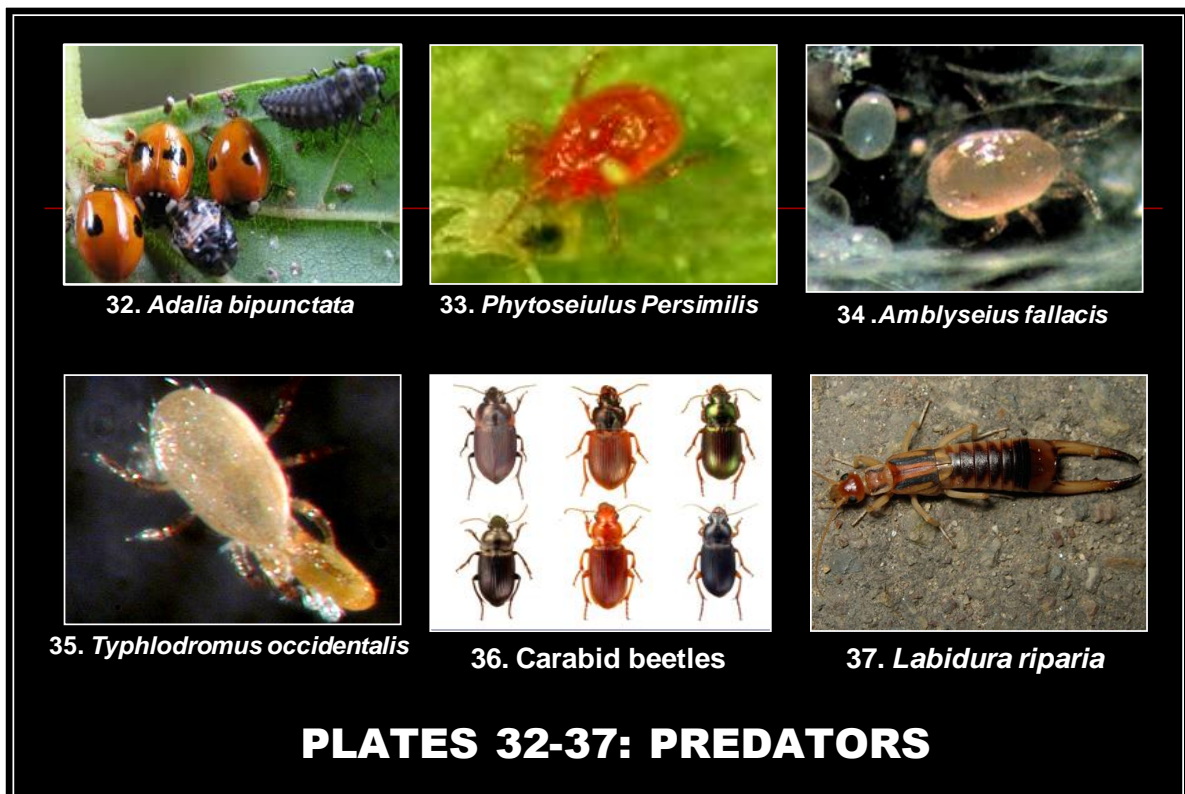


30. *Chrysoperla carnea*



31. *Macrolophus caliginosus*

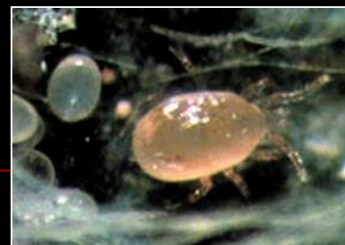
PLATES 23-31: PREDATORS



32. *Adalia bipunctata*



33. *Phytoseiulus Persimilis*



34. *Amblyseius fallacis*



35. *Typhlodromus occidentalis*



36. Carabid beetles



37. *Labidura riparia*

PLATES 32-37: PREDATORS