

Comparative studies on the effects of Bt-transgenic and non transgenic  
cotton on arthropod diversity, seed cotton yield and bollworms control

M.K. Dhillon <sup>1</sup>, and H.C. Sharma <sup>2</sup> \*

\*

<sup>1</sup> Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi-110 102, India

<sup>2</sup> International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru-502 324, India

\*Corresponding Author email : [h.sharma@cgiar.org](mailto:h.sharma@cgiar.org)

**Journal of Environmental Biology**

Vol. 34, 67-73, January 2013

This is author version post print archived in the official Institutional Repository of

ICRISAT [www.icrisat.org](http://www.icrisat.org)

---

**Comparative studies on the effects of *Bt*-transgenic and non-transgenic cotton  
on arthropod diversity, seedcotton yield and bollworms control**

**M.K. Dhillon<sup>1</sup> and H.C. Sharma<sup>2\*</sup>**

<sup>1</sup>Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi 110 102.

<sup>2</sup>International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

**Running title:** Arthropod diversity and toxin flow in insect fauna from *Bt*-cotton

Total no. of tables: **1**  
Total no. of figures: **6**

**\*Corresponding author**

Hari C. Sharma  
Principal Scientist – Entomology  
ICRISAT, Patancheru 502 324, Andhra Pradesh, India  
Ph.: +91 40 30713314  
Fax: +91 40 30713075  
E-mail: [h.sharma@cgiar.org](mailto:h.sharma@cgiar.org)

**Comparative study on the effects of *Bt*-transgenic and non-transgenic cotton on arthropod diversity, seedcotton yield and bollworms control**

**Abstract**

Keeping in view the need to assess bioefficacy and biosafety of *Bt*-transgenic cotton, we studied the effectiveness of commercial *Bt*-cotton in pest management, influence on arthropod diversity, natural enemies, and toxin flow in the insect fauna under field conditions. There were no significant differences in oviposition by *Helicoverpa armigera* on *Bt*-transgenic and non-transgenic cottons (9.2 versus 9.6 eggs plants<sup>-100</sup>), while the numbers of *H. armigera* larvae were significantly more on non-transgenic than on *Bt*-transgenic (10.4 versus 4.0 larvae plants<sup>-100</sup>) cotton. The *Bt*-cotton had significantly higher mature opened bolls (9.6 versus 4.4 bolls plant<sup>-1</sup>), lower bollworm damage (12.8 versus 40.2% bolls damaged), and higher seedcotton yield (667.7 versus 231.7 kg ha<sup>-1</sup>). Population of cotton leafhopper, *Amrasca biguttula biguttula* was lower (582.2 versus 732.2 leafhoppers plants<sup>-100</sup>), while that of whitefly, *Bemisia tabaci* was higher on *Bt*-transgenic (65.2 versus 45.6 whitefly plants<sup>-100</sup>) than on non-transgenic cotton. There was no significant influence of *Bt*-transgenic cotton on abundance of natural enemies of crop pests –

chrysopids (9.6 versus 8.4 chrysopids plants<sup>-100</sup>), ladybird beetles (16.0 versus 10.8 ladybirds plants<sup>-100</sup>), and spiders (128.4 versus 142.8 spiders plants<sup>-100</sup>). There were no significant differences in *H. armigera* (19.8 versus 20.9%), larval (7.4 versus 9.6%), and larval-pupal (1.3 versus 2.9%) parasitism on *Bt*-transgenic and non-transgenic cottons in the farmer's fields. The parasitism in larvae of *H. armigera* was far lower than that of the eggs, might be because of early mortality of *H. armigera* prior to parasitoid development in the host larvae. Although, Cry1Ac *Bt* toxin was detected in *Cheilomenes sexmaculatus*, chrysopids, *A. bigutulla bigutulla*, *Thrips tabaci*, *Myllocerus* sp., *Oxycarenus laetus*, *Dysdercus koenigii*, spiders, bugs, and grasshoppers, no significant differences were observed in their abundance on *Bt*-transgenic and non-transgenic cottons, suggesting that there were no adverse effects of *Bt*-cotton on the arthropod diversity under field conditions.

**Key words:** Transgenics, arthropod diversity, cotton, non-target effects, toxin flow, risk assessment.

## **Introduction**

Genetically modified plants expressing *Bacillus thuringiensis* (*Bt*) genes have been developed in different crops for resistance to insect pests, and some of them have been deployed successfully on a commercial scale for pest control (Hilder and Boulter, 1999; Sharma et al., 2004; James, 2007). Transgenic cotton and maize cultivars with resistance to lepidopteran insects have been released for cultivation in several countries, and were grown on more than 23 million ha worldwide in 2005 (James, 2005). Crop area under transgenic crops is increasing at a fast rate, and has reached to 134 million ha, of which transgenic crops with resistance to insect pests

constituted >40 million ha during 2009 (James, 2009). So far *Bt*-cotton has been commercialized in USA (1996), Mexico (1996), Australia (1996), China (1997), Argentina (1998), South Africa (1998), Colombia (2002), India (2002), Brazil (2005), and Burkina Faso (2008), and has occupied 49% of the total global cotton area (James, 2009). India rank first in the world occupying 8.4 m ha area under *Bt*-cotton (87% of world total cotton area) followed by China (5.4 m ha) (James, 2009). Considerable information has been generated on the relative efficacy of transgenic cottons against the target insect pests and the non-target effects in USA, Australia, and China (Wilson et al., 1992; Benedict et al., 1996; Ni et al., 1996; Cui and Xia, 1999; Greenplate, 1999; Guo et al., 1999; Gore et al., 2001; Fitt, 2003; Naranjo, 2005a, b; Torres and Ruberson, 2007), but little information is available on the effect of transgenic cottons on arthropod biodiversity in the tropics, where the transgenic cultivars have been released for cultivation only recently (Qaim and Zilberman, 2003; Naranjo, 2009). The cropping systems in tropics are quite diverse, and consist of several crops that serve as alternate and collateral hosts of the major pest, *Helicoverpa armigera* (Hubner), and other non-target insect pests. Because of the multiplicity of crops and cropping systems (mono-, mixed-, inter-, relay-, and sequential-cropping systems), the performance and interactions of transgenic crops in different agro-ecosystems are likely to be quite complex. One of the major concerns of transgenic crops is their effects on the non-target organisms, and many of the predators and parasitoids in arable systems are sensitive to the changes in the environment. Therefore, the present studies were undertaken to compare the abundance and diversity of arthropods, *Bt*-toxin flow in the insect fauna, and *H. armigera* damage and seedcotton yield advantage in the *Bt*-transgenic and non-transgenic cottons under field conditions.

## **Materials and Methods**

The *Bt*-transgenic cotton hybrid (*Bt* Mech 12, expressing *cryIAc* gene transferred from Mon531 Event) and the non-transgenic counterpart (Non-*Bt* Mech 12), obtained from Mahyco Seeds Ltd., India, were grown under field conditions on deep black soils (Vertisols) during the 2005 and 2006 rainy seasons at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. The seeds of each *Bt*-transgenic and non-transgenic cotton were sown on ridges 75 cm apart, and spaced at 50 cm on an area of 325 m<sup>2</sup>, in the last week of June, at the beginning of the rainy season. Both *Bt* and non-*Bt* cotton plots were divided in five subplots of 4 rows, 4 m long by leaving 4 m boundary all around for arthropod sampling. The crop was raised under rain-fed conditions. Normal agronomic practices were followed for raising the crop (basal fertilizer N: P: K:: 100: 40: 60 kg.ha<sup>-1</sup>). There was no insecticide application in the experimental plots during the crop-growing season.

### **Effects of *Bt*-transgenic and non-transgenic cotton on arthropod diversity and abundance of target and non-target insect pests**

The abundance of major insect pests - cotton bollworm [*H. armigera* (eggs and larvae)], cotton leafhopper [*Amrasca biguttula biguttula* (Ishida)], white fly [*Bemisia tabaci* (Gennadius)], ash weevils (*Mylloceris* spp.), cotton aphid (*Aphis gossypii* Glover); natural enemies – ladybird [*Cheilomenes sexmaculatus* (L.) (larvae + adults)], chrysopids (eggs + larvae), and spiders (*Clubiona* sp. and *Neoscona* sp.); and other less abundant arthropod species representing Hemiptera, Lepidoptera, Orthoptera, Hymenoptera and Coleoptera insect orders, were recorded on five *Bt*-transgenic and the non-transgenic cotton plants tagged at random in the middle two rows of sampling sub-plots as mentioned above, at five fortnightly intervals starting from 75

DAE (days after seedling emergence) to 135 DAE. The cotton leafhopper and white fly populations (adults and nymphs) were recorded on the undersurface of the top five fully expanded leaves of the same five tagged plants while, cotton aphid infestation was recorded as plants infested with aphids. The data on all the insect species were expressed as numbers plants<sup>-100</sup>. Total numbers of insect species recorded on the tagged *Bt*-transgenic and the non-transgenic cotton plants were considered to compute species richness. However, abundance of minor insect species representing hemipterans, lepidopterans, orthopterans, hymenopterans and coleopterans (excluding major insect pests and natural enemies mentioned above), on the tagged *Bt*-transgenic and the non-transgenic cotton plants were used to calculate Simpson's (1951) index of diversity..

### **Effect of *Bt*-transgenic and non-transgenic cotton on the activity and parasitism potential of parasitoids**

Natural *H. armigera* egg, larval, and larval-pupal parasitism was recorded on *Bt*-transgenic [RCH 2 BG1 and BGII, NCS 207 BGI, NCS 145 BGII (Bunny), Tulsi 118 BGII, MRC 7918 BGII, Ankur 5642 BGII] and the counterpart non-transgenic cottons at the farmer's fields from three states viz., Andhra Pradesh, Maharashtra, and Karnataka during the 2006 and 2007 cropping seasons. The egg samples both from *Bt* and the counterpart non-*Bt* cottons were collected in glass vials (15 ml capacity) from four different locations in each state. There were 250 eggs per location, consisting of 25 eggs per field from 10 randomly selected fields. The eggs were observed twice daily for emergence of any parasitoid, and the larvae hatched from these eggs were counted and removed from the sample vials. The data was expressed as percent egg parasitism. A total of 125 *H. armigera* larvae (2-3 instar stages) from five different locations in each state were collected in individual glass vials (50 ml capacity) from above mentioned *Bt* and

non-*Bt* cotton genotypes. The larvae were reared on respective foods till pupation, and the observations were recorded on larval or larval-pupal parasitization. The data was expressed as percent larval and larval-pupal parasitism.

### **Measurement of *Bt* toxin in insect fauna**

The insect species settled/visiting the *Bt*-transgenic and non-transgenic cotton were collected in 50 ml plexi glass vials, and kept in a deep freeze at  $-20^{\circ}\text{C}$ . About 25 to 50 mg of each insect species (number of specimens varied according to the insect size), were crushed (whole body) in PBS buffer in ratio of 1: 10 (insect sample: buffer) in Eppendorf tubes with a plastic pestle to detect the *Bt* proteins in the insect body using a double sandwich semi-quantitative ELISA (Agdia®) (Sharma et al., 2008). The negative and positive controls, and 0.5, 2.5, and 5.0 ppb *Bt* calibrators were run along with the test samples for the comparison of ELISA results.

### **Effects of *Bt*-transgenic and non-transgenic cotton on bollworm damage and seedcotton yield**

The observations on loss of squares and bolls of *Bt*-transgenic and non-transgenic cottons were recorded at 120 and 135 DAE in the *Bt*-transgenic and the non-transgenic cottons planted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The total numbers of squares and bolls fallen, and those damaged by bollworms [*H. armigera*, *E. vittella*, and *P. gossypiella*] were counted. Bollworms damage in *Bt*-transgenic and non-transgenic cotton was recorded by counting the numbers of damaged and undamaged mature bolls and green bolls on the same five plants in each observation plot. Seedcotton was picked-up manually from each plot, dried in the sun, and weighed.

## Statistical analysis

The data on the abundance of major target and non-target insect species were subjected to repeated measurements ANOVA (using REML analytical program in GENSTAT, 10.0 version), and the significance of differences were judged by  $\chi^2$  test. The cropping seasons and observation intervals were collectively considered as an environment factor in the data analysis. The significance of differences between the *Bt* and non-*Bt* cotton for insect damage and seedcotton yield were judged by F-test at  $P = 0.05$ , and the means were compared by least significant difference (LSD) at  $P = 0.05$ .

## Results and Discussion

There were no significant differences between *Bt*-transgenic and non-transgenic cotton for numbers of fallen squares ( $F = 1.48$ ;  $df = 1,4$ ;  $P = 0.583$ ). But, the fallen squares with bollworm damage were significantly more in non-transgenic than in the *Bt*-transgenic cotton ( $F = 12.99$ ;  $df = 1,4$ ;  $P = 0.023$ ). However, numbers of fallen bolls were significantly higher ( $F_{1,4} = 50.31$ ;  $P = 0.002$ ), and bollworm damaged bolls were lower ( $F = 13.93$ ;  $df = 1,4$ ;  $P = 0.02$ ) in *Bt*-transgenic than in the non-transgenic cotton hybrid (Fig. 1). Numbers of mature opened bolls were greater, and the bollworm damage was significantly lower (12.8 vs. 40.2%) in *Bt*-transgenic than in non-transgenic cotton (Fig. 2). However, the differences for total and bollworm damaged green bolls between the *Bt*-transgenic and non-transgenic cottons were nonsignificant at  $P = 0.05$ .

There were no significant differences in oviposition by *H. armigera* on *Bt*-transgenic and non-transgenic cottons ( $\chi^2 = 0.03$ ;  $P = 0.856$ ), while the numbers of eggs laid on cotton genotypes varied significantly across environments (seasons and observation intervals) ( $\chi^2 =$



13.02;  $P < 0.001$ ) (Table 1). The numbers of *H. armigera* larvae were significantly more on non-*Bt* than on *Bt*-transgenic cotton ( $\chi^2 = 10.24$ ;  $P = 0.001$ ). The variation in *H. armigera* larval density on *Bt* and non-*Bt* cottons was also significant across environments ( $\chi^2 = 2.53$ ;  $P = 0.007$ ). However, environment x genotypes and environment x replication interactions were nonsignificant.

There were significant differences in abundance of cotton leafhopper, *A. biguttula* between *Bt*-transgenic and non-transgenic cotton genotypes ( $\chi^2 = 11.45$ ;  $P < 0.001$ ), and environments ( $\chi^2 = 8.52$ ;  $P < 0.001$ ), while environment x genotypes and environment x replication interactions were nonsignificant. Significant differences were also recorded in abundance of white fly, *B. tabaci* between *Bt*-transgenic and non-transgenic cotton genotypes across environments ( $\chi^2 = 1.91$ ;  $P = 0.046$ ). The white fly population was greater on *Bt*-transgenic than on non-transgenic cotton. The differences in percent plants infested with aphid, *A. gossypii* between *Bt* and non-*Bt* cotton genotypes were nonsignificant (Table 1). There was no significant difference in abundance of ash weevils (*Mylloceris* spp.) on *Bt*-transgenic and non-transgenic cottons. However, their population varied significantly across environments ( $\chi^2 = 1.94$ ;  $P = 0.042$ ).

Species richness of plant inhabiting arthropod insects was similar for both *Bt*-transgenic and non-transgenic cotton, and a total of 24 insect species were observed for their relative abundance during each of 2005 and 2006 cropping seasons. The Simpson's index of diversity of minor insect species representing hemipterans, lepidopterans, orthopterans, hymenopterans and coleopterans ranged between 0.42 to 1.00 in *Bt*-transgenic and 0.52 to 1.00 in non-transgenic cotton. Simpson's index of diversity was lower for hemipterans, however for other insect orders it was close to unity (Fig. 3). This reduction in diversity index of hemipterans was largely due to

high numbers of neonate dusky cotton bug nymphs congregated on the tagged plants of either *Bt*-transgenic and/or non-transgenic cotton.

There were no significant differences in abundance of chrysopids ( $\chi^2 = 0.11$ ;  $P = 0.739$ ), ladybird ( $\chi^2 = 1.88$ ;  $P = 0.171$ ), and spiders ( $\chi^2 = 2.20$ ;  $P = 0.138$ ) on *Bt*-transgenic and non-transgenic cottons (Table 1). However numbers of ladybird ( $\chi^2 = 2.26$ ;  $P = 0.016$ ) and spiders ( $\chi^2 = 6.68$ ;  $P < 0.001$ ) varied significantly across environments.

Survey of *Bt*-transgenic and non-transgenic cottons for natural parasitization of eggs and larvae of *H. armigera* at farmer's fields from South-central India (Andhra Pradesh, Maharashtra and Karnataka) during the 2006 and 2007 cropping seasons revealed that there were no significant differences in *Helicoverpa* eggs (19.8 versus 20.9%), larval (7.4 versus 9.6%), and larval-pupal (1.3 versus 2.9%) parasitism on *Bt*-transgenic and non-transgenic cottons (Fig. 4). The parasitism in larvae of *H. armigera* was far lower than that of the eggs, might be because of early mortality of *H. armigera* prior to parasitoid development in the host larvae. *Trichogramma* spp. was identified as egg parasitoid, *Campoletis chlorideae* Uchida and *Eriborus* spp. as larval parasitoids, and *Stermiopsis* sp. and Syrphid fly as larval-pupal parasitoids on *H. armigera* in these regions. The results suggested that *Bt*-transgenic cottons are compatible with the egg parasitoid, *Trichogramma* spp.

The semi-quantitative ELISA of a total of 14 insect species [*Dysdercus koenigii* (Fab.), *Oxycaenus laetus* Kirby, 2 spider species (*Clubiona* sp., *Neoscona* sp.), *Cheilomenes sexmaculatus* (Fab.), *Myllocerus* sp., *A. bigutulla bigutulla*, one katydid species, bark mimicking grasshopper, dragon fly, and 4 different species of bugs] each from *Bt*-transgenic and non-transgenic cottons during the 2005 cropping season revealed that all the insect species (except one bug species) collected from *Bt*-transgenic cotton had *Bt*-toxin in their bodies. *Myllocerus* sp.,

*D. koenigii*, *O. laetus*, spiders, katydid, and bark mimicking grasshopper had >5.0 ppb, while *C. sexmaculatus*, *A. bigutulla bigutulla*, and the bugs had 2.5 to 5.0 ppb *Bt*-toxin in their bodies. However, none of the insect species collected from non-transgenic cotton had *Bt*-toxin.

A total of 16 insect species were collected from *Bt*-transgenic and non-transgenic cottons during the 2006 cropping season, and tested for the presence of *Bt*-toxin (Fig. 5). Amongst these *Clubiona* sp., short horned grasshopper, green grasshopper, blister beetle, *O. laetus*, *Myloccerus* sp., *A. bigutulla bigutulla*, *Thrips tabaci* Lindeman, chrysopid larvae, and one katydid species had >5.0 ppb, while *H. armigera* larvae and *C. sexmaculatus* adults and larvae had 2.5 to 5.0 ppb *Bt*-toxin. However, no *Bt*-toxin was detected in *Nezara viridula* (Linn.), *A. gossypii*, damselfly, and one bug species collected from *Bt* cotton. None of the insect species collected from non-transgenic cotton had *Bt*-toxin.

There were significant differences in seedcotton yield between *Bt*-transgenic and non-transgenic cotton ( $\chi^2 = 4.16$ ;  $P = 0.041$ ), and across environments ( $\chi^2 = 49.5$ ;  $P < 0.001$ ). Seedcotton yield was 667.7 kg.ha<sup>-1</sup> in *Bt*-transgenic compared to 231.7 kg.ha<sup>-1</sup> in non-transgenic cotton (Fig. 6).

Deployment of transgenic insect-resistant crops has made a significant contribution in reducing the dosage and frequency of insecticide application, and reduced yield losses due to insect pests (Brooks and Barfoot, 2008). Transgenic cotton with *Bt* genes in combination with insecticides are highly effective for bollworm control, even at lower rates of insecticide application (Brickle et al., 1999; Fitt, 2008). Transgenic cottons in combination with insecticides result in greater seedcotton yield (Sharma and Pampapathy, 2006). In the present studies, *Bt*-transgenic cotton had more mature opened bolls, lower bollworm damaged bolls, and had higher seedcotton yield, even without insecticide application.

Although, the numbers of eggs laid by *H. armigera* varied significantly across environments, there were no significant differences between *Bt*-transgenic and non-transgenic cottons. Sharma and Pampapathy (2006) reported higher numbers of *H. armigera* eggs and lower number of larvae on *Bt* than on non-*Bt* cottons, which might be because of better canopy due to lower damage by the target and non-target insects under protected conditions. There was no evidence of increased susceptibility or resistance of the transgenic *Bt* cottons to cotton leafhopper, *A. biguttula biguttula*, and the serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Sharma and Pampapathy, 2006). However, abundance of cotton leafhopper was lower, and of white fly was greater on *Bt*-transgenic than on non-transgenic cotton during the present studies. This may be because of the glabrous nature of the hybrid tested in the present studies, which is more susceptible to sucking pests (Sharma and Agarwal, 1983). There were no significant differences between *Bt* and non-*Bt* cotton plants infested with aphid, *A. gossypii*, and the numbers of ash weevils, *Myloccerus* spp. harboring *Bt*-transgenic and non-transgenic cotton.

The effects of transgenic crops on the natural enemies vary across crops and the cropping systems (Sharma and Ortiz, 2000; Shelton et al., 2002). Some of the variation may be due to differences in pest abundance between the transgenic and the non-transgenic crops. Although, several studies have suggested an expected reduction in parasitism by specialists of *Bt*-targeted pests probably due to host reduction, but no influence of *Bt* crops on the biological control by generalist predators have been evident, and *Bt* crops appear compatible with biological control within an IPM framework (Flint et al., 1995; Luttrell et al., 1995; Sims, 1995; Wang and Xia, 1997; Shelton et al., 2002; Romeis et al., 2008). From our studies under farmer's field conditions, it was also evident that there were no significant differences in natural parasitism of *H. armigera* eggs and larvae on *Bt*-transgenic and non-transgenic cottons. However, the

parasitism in larvae of *H. armigera* was much lower than that of the eggs, might be because of early mortality of *H. armigera* prior to parasitoid development in the host larvae. Although, the numbers of chrysopids, ladybird, and spiders varied across environments, no significant differences were found between *Bt* and non-*Bt* cottons during the present studies. Similar results on the relative abundance of these generalist predators in *Bt*-transgenic and non-transgenic cottons have been reported earlier under insecticide protected conditions (Sharma and Pampapathy, 2006; Sharma et al., 2007).

Total numbers of insect arthropods recorded on *Bt*-transgenic and non-transgenic cotton were similar. Also, the Simpson's index of diversity of minor insect species was unity, except for hemipterans, which might be largely due to high numbers of neonate dusky cotton bug nymphs congregated on the tagged plants of either *Bt*-transgenic and/or non-transgenic cotton. *Bt* toxins were detected in insects collected from *Bt*-transgenic cotton, but there were no significant differences in the numbers of canopy dwelling insect species on *Bt*-transgenic and non-transgenic cotton. Several studies reviewed through meta-analysis (Naranjo, 2009) have suggested that the effects of *Bt* crops on non-target invertebrates are minimal, if any, are much lower in comparison with alternative pest control measures such as broad-spectrum insecticides. Though American and spotted bollworms have been relegated to secondary pest status, pink bollworm and *Spodoptera litura* (Fab.) are assuming serious proportions after introduction of *Bt* cotton. The reduction in insecticide sprays, especially during flowering and boll formation phases, has resulted in resurgence of mealy bugs, *Phenacoccus solenopsis* (Tinsley) and *Maconellicoccus hirsutus* (Green); aphids, *Aphis gossypii* Glover; thrips, *Thrips tabaci* Lindeman; and becoming major sucking pests of cotton in India (Sharma et al., 2005; Karihaloo and Kumar, 2009; Nagrare, et al., 2009). The cropping systems and biodiversity in different

agroecosystems are quite diverse worldwide, and the breadth of coverage of biodiversity within agroecosystems in general and arthropods in particular for biosafety studies is not sufficient under tropics, therefore, there is a continued need to monitor the effects of *Bt*-transgenic crops on the abundance and diversity of arthropods.

### **Acknowledgements**

The authors thank the staff of entomology for their help in data recording, and funding by Indo-Swiss Collaboration on Biotechnology (ISCB), Swiss Agency for Development and Cooperation (SDC), Berne, Switzerland, Department of Biotechnology (DBT), and Department of Science and Technology (DST), New Delhi, India, is gratefully acknowledged.

### **References**

- Benedict, J.H., Sachs, E.S., Altman, D.W., Deaton, D.R., Kohel, R.J., Ring, D.R. and Berberich, B.A.: Field performance of cotton expressing Cry IA insecticidal crystal protein for resistance to *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **89**, 230-238 (1996).
- Brickle, D.S., Turnipseed, S.G., Sullivan, M.J. and Dugger, P.: The efficacy of different insecticides and rates against bollworms (Lepidoptera: Noctuidae) in B.T. and conventional cotton. In: Proceedings, Beltwide Cotton Production and Research Conference, 3-7 Jan 1999, Orlando, Florida, USA, vol. 2 (Ed.: D. Richter). National Cotton Council, Memphis, USA. pp. 934-936 (1999).
- Brookes, G. and Barfoot, P.: Global impact of biotech crops: socio-economic and environmental effects 1996–2006. *AgBioForum* **11**, 21–38 (2008).

- Cui, J.J. and Xia, J.Y.: Effects of transgenic Bt cotton on development and reproduction of cotton bollworm. *Acta Agri. Univ. Henan* **33**, 20-24 (1999).
- Fitt, P.: Deployment and impact of transgenic Bt cottons in Australia. In: *The Economic and Environmental Impacts of Agbiotech: A Global Perspective* (Ed.: N.G. Kalaitzandonakes). Kluwer Academic Press, New York, USA. pp. 141-164 (2003).
- Fitt, G.P.: Have Bt crops led to changes in insecticide use patterns and impacted IPM? In: *Integration of Insect-Resistant Genetically Modified Crops with IPM Systems* (Eds.: J. Romeis, A.M. Shelton, G.G. Kennedy). Springer, Berlin, Germany. pp. 303–328 (2008).
- Flint, H.M., Henneberry, T.J., Wilson, F.D., Holguin, E., Parks, N. and Buehler, R.E.: The effects of transgenic cotton, *Gossypium hirsutum* L.; containing *Bacillus thuringiensis* toxin genes for the control of the pink bollworm, *Pectinophora gossypiella* (Saunders) Lepidoptera, Gelechiidae and other arthropods. *Southwest. Entomol.* **20**, 281-292 (1995).
- Gore, J., Leonard, B.T. and Adamczyk, J.J.: Bollworm (Lepidoptera: Noctuidae) survival on Bollgard and Bollgard II cotton flower bud and flower components. *J. Econ. Entomol.* **94**, 1445-1451 (2001).
- Greenplate, J.T.: Quantification of *Bacillus thuringiensis* insect control protein Cry1A(c) over time in Bollgard cotton fruit and terminals. *J. Econ. Entomol.* **92**, 1377-1383 (1999).
- Guo, S.D., Cui, H.Z., Xia, L.Q., Wu, D.L., Ni, W.C., Zhang, Z.L., Zhang, B.L. and Xu, Y.J.: Development of bivalent insect-resistant transgenic cotton plants. *Sci. Agri. Sinica* **32**, 1-7 (1999).
- Hilder, V.A. and Boulter, D.: Genetic engineering of crop plants for insect resistance - a critical review. *Crop Prot.* **18**, 177-191 (1999).

- James, C.: Global status of commercialized biotech/GM crops: 2005. ISAAA Brief No. 34, International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY, USA (2005).
- James, C.: Global status of commercialized biotech/GM crops: 2007. ISAAA Brief No. 37. International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY, USA (2007).
- James, C.: Global status of commercialized biotech/GM crops: 2009. ISAAA Brief No. 39. International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY, USA (2009).
- Karihaloo, J.L. and Kumar, P.A.: Bt cotton in India – A status report (Second Edition). Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB), New Delhi, India. 56 pp. (2009).
- Luttrell, R.G., Mascarenhas, V.J., Schneider, J.C., Parker, C.D. and Bullock, P.D.: Effect of transgenic cotton expressing endotoxin protein on arthropod population in Mississippi cotton. In: Proceedings, Beltwide Cotton Production Research Conference. National Cotton Council of America, Memphis, TN, USA. pp. 760-763 (1995).
- Nagrare, V.S., Kranthi, S., Biradar, V.K., Zade, N.N., Sangode, V., Kakde, G., Shukla, R.M., Shivare, D., Khadi, B.M. and Kranthi, K.R.: Widespread infestation of the exotic mealybug species, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae), on cotton in India. *Bull. Entomol. Res.* **99**, 537-541 (2009).
- Naranjo, S.E.: Long-term assessment of the effects of transgenic *Bt* cotton on the abundance of nontarget arthropod natural enemies. *Environ. Entomol.* **34**, 1193-1210 (2005a).



- Naranjo, S.E.: Long-term assessment of the effects of transgenic *Bt* cotton on the function of the natural enemy community. *Environ. Entomol.* **34**, 1211-1223 (2005b).
- Naranjo, S.E.: Impacts of Bt crops on non-target invertebrates and insecticide use patterns. *CAB Reviews: PAVSNNR* **4(011)**, 1-23 (2009).
- Ni, W.C., Huang, J.Q., Guo, S.D., Shu, C.G., Wu, J.Y., Wang, W.G., Zhang, Z.L., Chen, S., Mao, L.Q., Wang, Y., Xu, Y.J., Gu, L.M., Zhou, B.L., Shen, X.L. and Xiao, S.H.: Transgenic bollworm-resistant cotton plants containing the synthetic gene coding *Bacillus thuringiensis* insecticidal protein. *Jiangsu J. Agril. Sci.* **12**, 1-6 (1996).
- Qaim, M. and Zilberman, D.: Yield effects of genetically modified crops in developing countries. *Science* **299**, 900-902 (2003).
- Romeis, J., Van Driesche, R., Barratt, B. and Bigler, F.: Insect-resistant transgenic crops and biological control. In: Integration of Insect-Resistant Genetically Modified Crops with IPM Systems (Eds. J. Romeis, A.M. Shelton and G.G. Kennedy). Springer, Berlin, Germany. pp. 87-117 (2008).
- Sharma, H.C. and Agarwal, R.A.: Role of some chemical components and leaf hairs in varietal resistance in cotton to jassid, *Amrasca biguttula biguttula* Ishida. *J. Entomol. Res.* **7**, 145-149 (1983).
- Sharma, H.C., Arora, R. and Pampapathy, G.: Influence of transgenic cottons with *Bacillus thuringiensis cryIAc* gene on the natural enemies of *Helicoverpa armigera*. *BioControl* **52**, 469-489 (2007).
- Sharma, H.C., Dhillon, M.K. and Arora, R.: Effects of *Bacillus thuringiensis*  $\delta$ -endotoxin-fed *Helicoverpa armigera* on the survival and development of the parasitoid *Campoletis chloridae*. *Entomol. Exp. Appl.* **126**, 1-8 (2008).

- Sharma, H.C. and Pampapathy, G.: Influence of transgenic cottons on the relative abundance and damage by target and non-target insect pests under different protection regimes in India. *Crop Prot.* **25**, 800-813 (2006).
- Sharma, H.C. and Ortiz, R.: Transgenics, pest management, and the environment. *Curr. Sci.* **79**, 421-437 (2000).
- Sharma, H.C., Sharma, K.K., Seetharama, N. and Crouch, J.H.: Genetic engineering of crops for insect control: Effectiveness and strategies for gene deployment. *Crit. Rev. Plant Sci.* **23**, 47-72 (2004).
- Sharma, O.P., Bambawale, O.M., Dhandapani, A., Tanwar, R.K., Bhosle, B.B., Lavekar, R.C. and Rathod, K.S.: Assessment of severity of important diseases of rainfed *Bt* transgenic cotton in Southern Maharashtra. *Indian Phytopathol.* **58**, 483-485 (2005).
- Shelton, A.M., Zhao, J.Z. and Roush, R.T.: Economic, ecological, food safety, and social consequences of the deployment of *Bt* transgenic plants. *Ann. Rev. Entomol.* **47**, 845-881 (2002).
- Simpson, E.H.: The interpretation of interaction in contingency tables. *J. Royal Stat. Sci.* **13B**, 238-241 (1951).
- Sims, S.R.: *Bacillus thuringiensis* var. *kurstaki* (CryIA (c) protein expressed in transgenic cotton: effects on beneficial and other non-target insects. *Southwest. Entomol.* **20**, 493-500 (1995).
- Torres, J.B. and Ruberson, J.R.: Abundance and diversity of ground-dwelling arthropods of pest management importance in commercial *Bt* and non-*Bt* cotton fields. *Ann. Appl. Biol.* **150**, 27-39 (2007).

Wang, C.Y. and Xia, J.Y.: Differences of population dynamics of bollworms and of population dynamics of major natural enemies between Bt transgenic cotton and conventional cotton.

*China Cottons* **24**, 13-15 (1997).

Wilson, W.D., Flint, H.M., Deaton, R.W., Fischhoff, D.A., Perlak, F.J., Armstrong, T.A., Fuchs, R.L., Berberich, S.A., Parks, N.J. and Stapp, B.R.: Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm (Lepidoptera: Gelechiidae) and other insects.

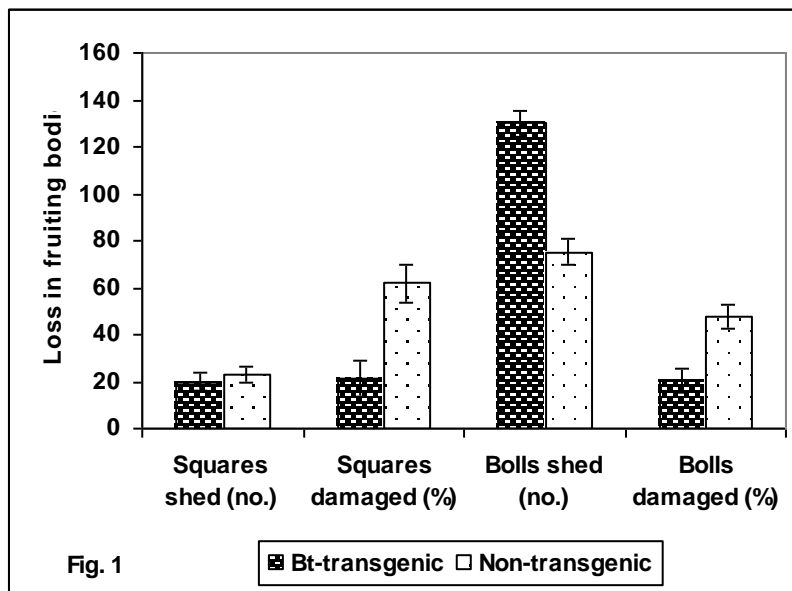
*J. Econ. Entomol.* **85**, 1516-1521 (1992).

Table 1. Abundance of major target and non-target insect pests, and natural enemies in *Bt*-transgenic and non-transgenic cotton

Arthropod species	Number of insects plants <sup>-100*</sup>	
	<i>Bt</i> -transgenic	Non-transgenic
<b>Target insect pest</b>		
<i>Helicoverpa armigera</i> eggs	9.2 ± 2.2	9.6 ± 2.2
<i>Helicoverpa armigera</i> larvae	4.0 ± 2.0	10.4 ± 2.0
<b>Non-target insect pests</b>		
Jassids, <i>Amrasca biguttula biguttula</i>	582.2 ± 44.4	732.2 ± 44.4
White fly, <i>Bemisia tabaci</i>	65.2 ± 8.0	45.6 ± 8.0
<i>Aphis gossypii</i> infested plants (%)	2.0 ± 1.1	4.4 ± 1.1
Ash weevils, <i>Mylocerus</i> spp.	21.6 ± 4.6	15.2 ± 4.6
<b>Natural enemies</b>		
Ladybird, <i>Cheilomenes sexmaculatus</i>	16.0 ± 3.8	10.8 ± 3.8

Lacewings	9.6 ± 3.6	8.4 ± 3.6
Total spiders, <i>Clubiona</i> sp., <i>Neoscona</i> sp.	128.4 ± 9.8	142.8 ± 9.8

\* = Values in the table are means across replications, observation intervals, and seasons.



**Fig. 1. Square and boll shedding (mean ± SE) in *Bt*-transgenic and non-transgenic cotton due to insect pests, and/or physiological factors.**

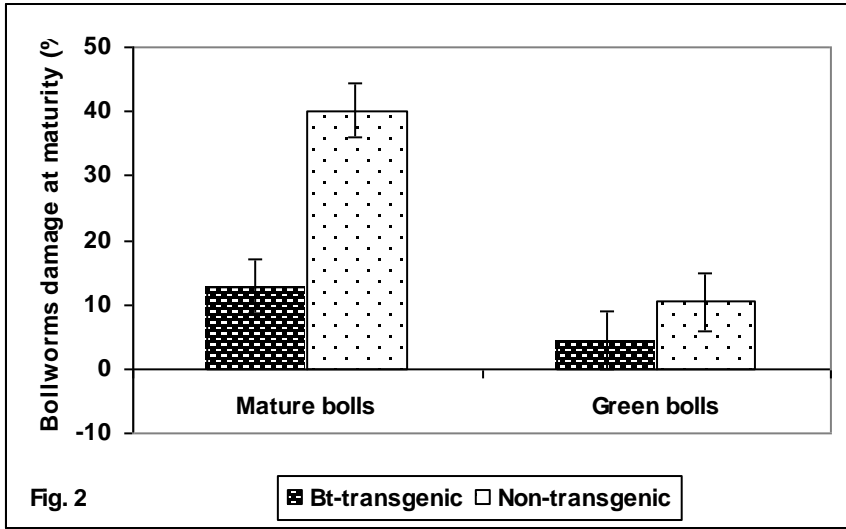


Fig. 2. Bollworms damage (mean  $\pm$  SE) in *Bt*-transgenic and non-transgenic cotton at maturity.

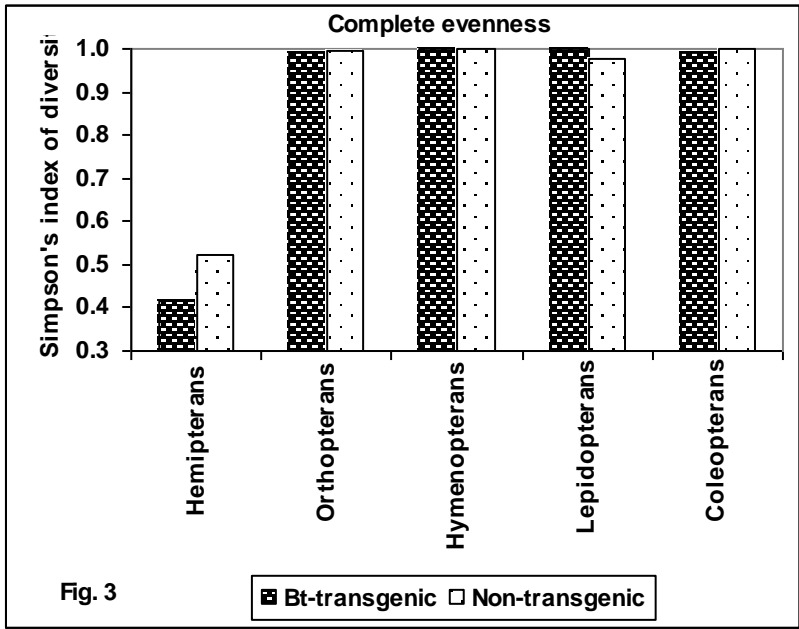


Fig. 3. Simpson's index of diversity for arthropods inhabiting *Bt*-transgenic and non-transgenic cotton.

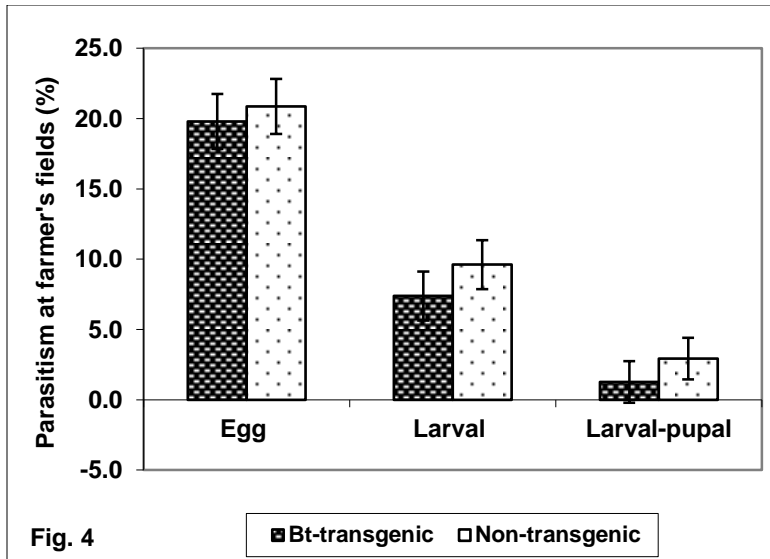
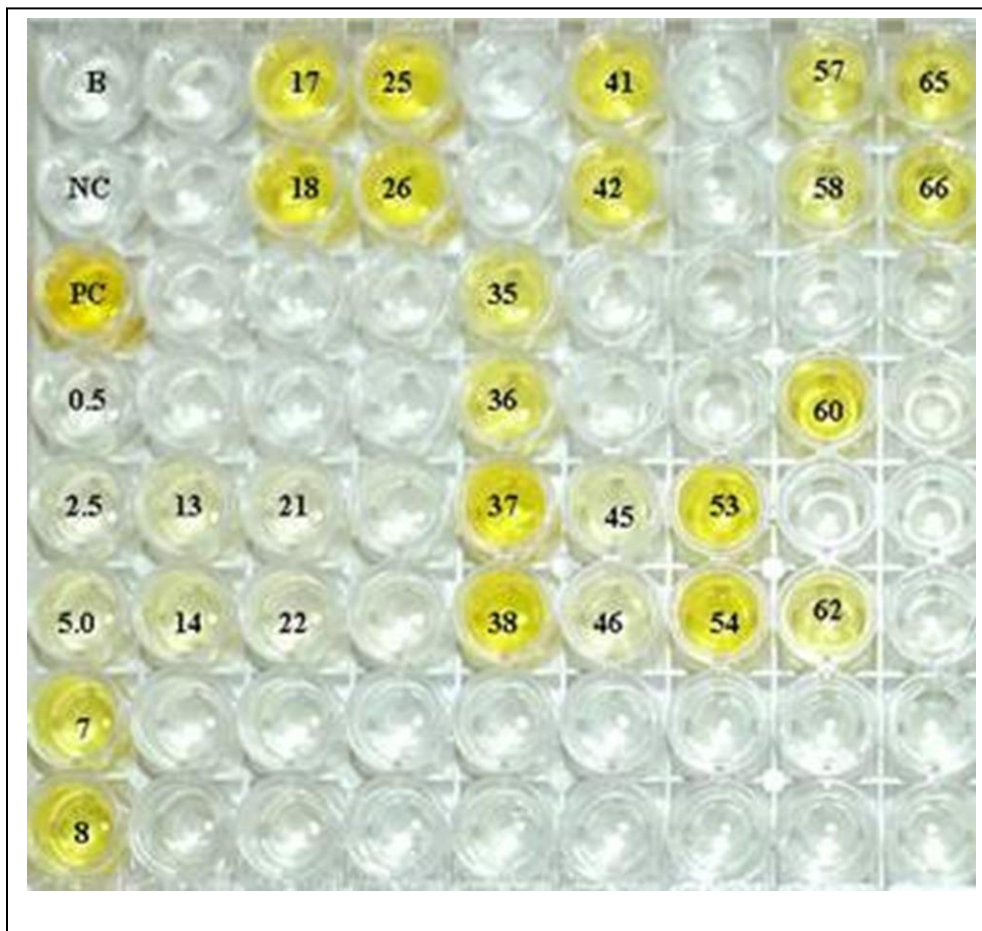
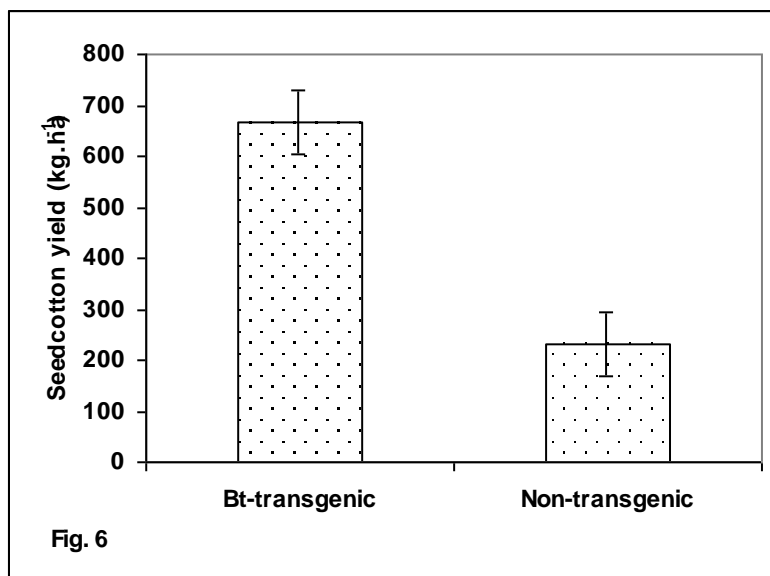


Fig. 4. *Helicoverpa armigera* egg, larval, and larval-pupal parasitism (mean  $\pm$  SE) in *Bt*-transgenic and non-transgenic cottons under farmer's field conditions.



**Fig. 5. *Bt*-toxin flow from transgenic cotton in insect fauna through different trophic levels.**

Insect species imbibed *Bt* toxin from *Bt*-transgenic cotton: 7,8: blister beetle; 13,14: spiders; 17,18: short horned grasshopper; 21,22: *Helicoverpa* larvae; 25,26: green grasshopper; 35,36: *A. biguttula biguttula*; 37,38: *O. laetus*; 41,42: *Mylocerus* spp.; 45,46: *C. sexmaculatus* adult; 53,54: cotton leafhopper; 57,58: *Thrips tabaci*; 60: chrysopid larvae; 62. *C. sexmaculatus* larvae; 65,66: katydids.



**Fig. 6. Seedcotton yield (mean  $\pm$  SE) in *Bt*-transgenic and non-transgenic cotton under unprotected conditions.**