2207 Resistance of Transgenic Bt plus CpTI Cotton to *Helicoverpa armigera* Hubner and its Effect on other Insects in China

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China is the largest cotton-producer in the World with about 4.5 million tons of lint produced annually, which accounts for over 20% of the World's total production (Xia *et al.* 2001). Damage from pests is one of the major limiting factors for cotton production in China, among which cotton bollworm (*Helicoverpa armigera* Hübner) is a key one (Fang *et al.* 1992). In the early 1990s, due to the dramatic changes in cotton cropping systems, favorable weather condition and rapid development of insecticide resistance, there had been a serious bollworm outbreak in China, resulting in losses of 15-30% cotton lint (Xia, 1993, 1994, 1997). Cultivation of insect-resistant cultivars proves an economical and feasible way for bollworm control.

With the rapid development and wide application of modern biotechnology, a great achievement has been made in inserting insect-resistant genes from other organisms into cotton. Scientists at Agracetus in USA were the first to genetically engineer cotton plants expressing the δ-endotoxin gene from *Bacillus thruingiensis* (Bt) var.kurstaki (Umbeck *et al.* 1987). At the same time, scientists at Monsanto in USA developed transgenic cotton plants containing Bt genes CryIA (b) from bacterial strain HD-1 and CryIA (c) from HD-73 (Perlak *et al.* 1990). These transformed plants were effective in controlling important lepidopterous cotton pests in laboratory, field-cage and open-field evaluations (Benedict *et al.* 1993, 1996; Fitt *et al.* 1994; Genkins *et al.* 1993; Halcomb *et al.* 1996; Wilson *et al.* 1992).

Since the early 1990s, the Chinese scientists have been working on genetically engineering cotton and have successfully transformed the Bt gene *cry1Ac* into the Chinese cotton plants (Cui and Guo, 1995; Ni *et al.* 1998; Xia, 1996; Xia *et al.* 1995; Xie *et al.* 1991). For the resistance management of cotton bollworm to transgenic Bt cotton, the Chinese scientists have also successfully inserted the Bt plus CpTI (Cow pea Tripsin Inhibitor) gene into the Chinese cotton plants (Cui, 2003; Li *et al.* 2000, 2005). So far, over 20 transgenic cultivars with gene of Bt or Bt Plus CpTI have been bred and released in production, demonstrating a high resistance to cotton bollworm (Cui and Guo, 1995; Ni *et al.*1998; Xia *et al.*1995b). Extension of such transgenic cotton cultivars has brought about significant economic, social and ecological benefits (Xia *et al.* 2006).

Xia *et al.* (2002) studied in details the resistance of transgenic Bt cotton to *H. armigera* and its effects on other insects in China. In the present paper, we studied the resistance of transgenic Bt plus CpTI cotton to *H. armigera* and its effects on other insects in China. All studies were carried out in the China Cotton Research Institute (CCRI, Anyang, Henan, China) during 1996-2006. The cotton cultivars were CCRI 41 (transgenic Bt plus CpTI cotton) and CCRI 23 (non Bt cotton).

1 Resistance to Cotton Bollworms

1.1 Variation in resistance

The resistance of CCRI 41 to *H. armigera* was studied in laboratory and field experiments. The resistance was evaluated in terms of the bionomic responses to feeding on various structures of host plants at different times of the cropping season, and to various bollworm instars. Bollworms were fed for 5 days in the laboratory with six structures of the transgenic Bt plus CpTI and non-transgenic plants (leaves, bracts, squares without bracts, petals, flowers without bracts and petals, and small bolls without bracts) in June, July, August and September. We also reared the 1st through 6th bollworm instars with six plant structures for 5 days in the laboratory. The field experiment was carried out in an insecticide-free field (2 ha) with Bt plus CpTI and non-transgenic plants. Every 5 days from June to September, we inspected the number of bollworm eggs and larvae from 10 randomly selected plots (each with 10 plants).

Evaluation in the laboratory indicated CCRI 41 was effective in killing H. *armigera* young larvae, whereas the levels of resistance varied with the crop stage, plant structure and larval age (size) (Table 1 and 2). The mortality of bollworm young larvae fed six structures of the transgenic plants was all significantly higher ($P<0.05$) than on the respective controls. Thus, the corrected mortality of young larvae fed each structure was computed for comparison, using the formula of Abbott (1925). Clearly, the resistance of transgenic plants to bollworm young larvae decreased with crop senescence, where the mean-corrected mortality over six structures was 98.5% in June, 85.0% in July, 87.7% in August, and 80.4% in September (Table 1). The season mean-corrected mortality of the young larvae was higher on bracts and squares than on the leaves. Some researchers have observed a decreasing level of *H. armigera* resistance in transformed Bt cottons with crop senescence (Fitt et al. 1994; Xia *et al.* 1995b; Zhao *et al*. 1998a), and a low level of bollworm resistance on petals and flowers in transformed Bt cottons (Dong *et al.* 1997b; Zhao *et al.* 1998a).

The resistance of transgenic cotton to bollworms decreased with larval age (Table 2). Averaged over six structures, the mean-corrected mortality of the 1st to 6th instars was approximately 85%, 80%, 70%, 60%, 30%, and 10%, respectively. These results were consistent with those observed in other transgenic Bt cultivars resistant to *H. armigera, H. Punctigera* (Wallengren), *H. zea* (Boddie) and *H. virescens* (Dong *et al.* 1997b ; Fitt *et al.* 1994; Halcomb *et al.* 1996; Xia *et al.* 1999; Zhao *et al.* 1998b).

Evaluation in field experiments showed that the transgenic Bt plus CpTI plants had little influence on the number of bollworm eggs laid on them, but greatly suppressed the larval populations in all three generations, thereby reducing their damage from the insect. The season-mean number of eggs laid on CCRI 41 in the field, over each sampling date, was only slightly lower (0.27 per plant) than on the non-transgenic control (0.32 per plant). The peak density of bollworm larvae on the non-transgenic plants for the 1st to 3rd generation in the field cage was significantly greater (1.21, 0.37 and 0.46 per plant, respectively) than on the transgenic plants (0.14, 0.09 and 0.17 per plant, respectively). Likewise, the season-mean number of larvae on the non-transgenic plants in the field, over each sampling date, was about 5 times that on the transgenic plants. The percentage of injured terminals and squares plus boll on the non-transgenic control in the field, averaged over three generations, was significantly greater (46-91%) than on the transgenic plants (16-18%). Field evaluations in USA and Australia demonstrated a similar efficacy of transgenic Bt cottons in suppressing populations of *H.virescens*, *H. zea*, *H. armigera*, *H. punctigera* and *PectinoPhora gossypiella* (Saunders) (Benedict *et al*.1993; Fitt *et al*. 1994; Sachs *et al*.1996; Wilson *et al*.1992).

1.2 Effect on bionomics

The resistance mechanism of CCRI 41 to *H. armigera* was studied with respect to effects on insect bionomics. We continuously reared each larval instars until death with the six plant structures in the laboratory, to determine their effects on survival, growth and reproductive potential of *H. armigera.*

Transgenic Bt plus CpTI cotton adversely affect development, survival and reproductive potential of *H. armigera*. The weight of larvae, larval duration, pupated rate, weight of pupae, pupae duration, molting rate and lifespan of adult of bollworms, fed various structures of Bt plus CpTI cotton, were all decreased, compared with the non-transgenic control (Tables 3). No larvae of the 1st to 4th instars, fed leaves, bracts, squares, petals and flowers of CCRI 41， could survive to pupation. The duration of 6th instars on all tested structures of the transgenic Bt plus CpTI cotton was significantly longer than on the non-transgenic control. The weight of 6th instars fed all structures of the transgenic Bt plus CpTI cotton was less than that on the respective control.

It was commonly observed that the 1st to 4th instars of *H. armlgera, H. zea* and *H. virescens* larvae fed transgenic Bt cotton could not survive to pupation (Halcomb *et al*. 1996; Zhao *et al.* 1998b). A decrease in development and survival rates of those lepidopterous immatures has been reported in other transgenic Bt cotton (Benedict *et al*.1992, 1993; Dong *et al.*1997b; Fitt *et al*. 1994; Holcomb *et al*.1996; Jenkins *et al*. 1993; Zhao *et al.* 1998b) and Bt diets (Could *et al.* 1991), though little has been known about their effects on the insect reproductive potential.

2 Effects on Other Herbivores

2.1 Effects on other lepidopterous pests

Except for the main target, *H. armigera*, there are several other lepidopterous pests frequently causing damage to cotton, such as cut worm (*Agrotis ipsilon* Hüfnagel), corn borer (*Ostrinia furnacalis* Hübner) and small looper (*Anomis flava* Fabricius) (Fang *et al.* 1992). We evaluated the resistance of CCRI 41 to *A. ipsilon* in the laboratory, by feeding their neonates until death with the young leaves from field-grown transgenic Bt plus CpTI and non-transgenic plants.

Laboratory evaluation revealed detrimental effects of CCRI 41 on the survival and growth of cutworms (Table 4). The percentage of cutworm larvae surviving to pupation and then to the adults (25.6%) on the transgenic Bt plus CpTI (30.0% and 32.2%, respectively) was significantly lower than on non-transgenic control (85.0% and 75.0%, respectively). Compared with the non-transgenic control, the cutworm larval weight (at day 6) and pupa weight at day 6 on the transgenic plants was decreased. These results are consistent with those in transgenic Bt cotton (Cui *et al.* 2002; Xia *et al.* 2001).

2.2 Effects on non-lepidopterous pests

Besides the lepidopterous pests, there are several sucking pests injurious to cotton, such as cotton aphid (*Aphis gossypiii* Glover), red spider mite (*Tetranychus cinnabarinus* Boisduval) and thrips (*Thrips tabaci* Lindeman). We sampled *A. gossypii* and *T. cinnabarinus* on each phenotypic cotton from randomly selected 10 plots (each with 10 plants) in an unsprayed field (1.5 ha), every 5 days from April to September.

Compared to the non-transgenic control, abundance of cotton aphid (Fig.1) and red spider mite (Fig.2) was increased on transgenic Bt plus CpTI plants. The season-mean population numbers of these two pests on the Bt plus CpTI plants, were increased by 21.6% and 158.3% at a higher peak density, respectively. These sucking pests have been also observed more abundant on transgenic Bt plants in single cotton and cotton / wheat intercropping systems (Cui, 1998; Cui and Xia, 1998; Xia et al. 1999). Wilson *et al.*（1992）reported higher populations of *Bemisia tabaci* (Gennadius) on the transgenic Bt plants than on the non-transgenic control. An increased abundance of sucking pests on transgenic Bt plus CpTI cottons may have been a consequence of reduced leaf feeding damage by lepidopterous insects rather than increased susceptibility of transformed plants to those pests (Cui, 1998; Cui and Xia, 1998; Wilson et al. 1992).

3 Effects on Major Predators

3.1 Effects on functional response

Several predators have been recorded to attack *H. armigera* larvae, such as *Propylaea japonica* Goeze, *Coccinella septempunctata* L.、*Orius minutus* L.and *Erigonidium graminicolum* Sundevall. We determined the effects of CCRI 41 on the functional responses of these four predators to *H. armigera* in the laboratory by rearing the field-collected adult predators (2-5 days old) with transgenic Bt plus CpTI cotton-fed and non-transgenic cotton-fed young larvae (prey), and then fitting the data with Holling's (1959) typeⅡ predation equation. For all predators tested, the prey density levels were 20, 40, 60, 80 and 120, each with 5 replicates.

Laboratory evaluation indicated an increase in maximum predation （Na）and search rates (a) but a decrease in handling time （Th）for all tested predators preying on the transgenic Bt plus CpTI cotton fed-prey in comparison with the Bt-free prey (Table 5). The handling time of *P. japonica* preying on the Bt plus CpTI cotton fed-larvae was decreased by over 3.8%, though it was decreased by less than 29.2 % for the non-transgenic cotton predators. The search rate of *E. graminicolum* preying on the transgenic Bt plus CpTI cotton-fed larvae was decreased on the Bt-free prey, while it was increased by 27.1 % for the non-transgenic cotton predators. *P. Japonica* was more effective in preying on the Bt plus CpTI cotton-fed bollworm larvae than the other predators because its maximum predation rate was more that on the control. Increased predation rate of those predators could be due to the smaller size and less vigor of the transgenic Bt plus CpTI cotton-fed prey. A similar trend has been also observed in transgenic Bt cotton (Cui, *et al.* 2005; Xia *et al.* 2001).

3.2 Effects on predator population dynamics

The population dynamics of *E. graminicolum* and *P. japonica* on transgenic Bt plus CpTI cotton was studied in field experiments. Every 5 days from April to September, the population numbers of these two predatory species on each phenotypic cotton were inspected from randomly selected 10 plots (each with 10 plants) in an insecticide free-field.

Field evaluation revealed an increased abundance of *E. graminicolum* (Fig.3) but a decreased abundance of *P.japonica* (Fig.4) on transgenic Bt plus CpTI cotton, compared with its parental cultivar. The season-mean numbers of *E. graminicolum* on transgenic Bt plus CpTI plants, compared with the transgenic Bt control, were increased by 4.5% with the highest peak density increased by 9.5%. The season-mean number of *P. japonica* on the transgenic plants was increased by by 7.5 % with its highest peak density increased by 8.9%, compared with the non-transgenic control. An increased abundance of *E. graminicolum* on the transgenic plants may have resulted from the increased populations of some sucking insects (prey), noticeably cotton aphid (Fig. 1). Reduced *P. japonica* populations on the transgenic plants may be ascribed to the dramatic decrease in bollworm larval populations (Fig.4) which this predator actively consumes (Table 5). Helbeck *et al.*（1998a, b） showed that the mean total immature mortality for *Chrysoperla carnea* Stephens, raised on the transgenic Bt corn-fed *Ostrinia nubilalis* (Hübner) and *Spodoptera Iittoralis* (Boisduval), was significantly higher than those raised on the Bt-free prey, and their development time was longer than that in control. They further observed that the cryIA (b) was toxic to *C. carnea* at 100 ug / ml of the encapsulated artificial diets.

4 Effects on Major Parasitoids

4.1 Effects on parasitism

More than 10 parasitoid Species have been known to attack the different life stages of lepidopterous pests in cotton (Fang *et al.* 1992). Two of them, *Microplitis* sp. and *Campoletis chlorideae* Uchida, are often found to parasitize *H.armigera* larvae with parasitization ranging from 2-36 % (Cui, 1998; Fang *et al* .1992). The effect of CCRI 41 on parasitism of these two parasitoids was evaluated in the laboratory, by rearing them with transgenic Bt plus CpTI cotton-fed and non-Bt fed bollworm young larvae (hosts).

Transgenic Bt plus CpTI cotton affected the survival and growth of *Microplitis* sp. and *C.chlorideae.* The percentage of parasitization and adult emergence, as well as the weight of cocoons and adults of both parasitoids reared with Bt plus CpTI cotton-fed bollworm young larvae, were all significantly decreased compared with the non-ransgenic control-fed hosts (Table 6). The mechanism for transgenic plants to affect parasitism of both larval parasitoids is similar to that of commercial Bt products as observed by (Salama *et al*. 1982; Qing, *et al*. 2004).

4.2 Effects on parasitoid population dynamics

Population dynamics of *Microplitis* sp in transgenic Bt plus CpTI cotton was studied in the field experiments. Its population numbers in each phenotypic cotton were observed from randomly selected 10 plots (each with 10 plants) in an insecticide-free field (2 ha), every 5 days from June to September.

Field observation showed a significantly reduced abundance of *Microplitis* sp. on CCRI 41, compared with the non-transgenic control (Fig. 5), as there were fewer larvae to parasitize. Thus, the season-mean number of *Microplitis* sp. on the non-transgenic control was 7-11 times that on the transgenic Bt plus CpTI plants. This evidence further indicates that transgenic plants exert a similar impact on bollworm larval parasitoids as commercial Bt products (see also Johnson and Gould, 1992).

5 Effects on Arthropod Community

5.1 effects on species composition

We evaluated the effect of transgenic Bt plus CpTI plants on arthropod com-mutinies in an unsprayed field, using the method described by Xia *et al.* (1995a). Field evaluation revealed no adverse effect of CCRI 41 on species composition of arthropod communities. The number of arthropod species on the transgenic Bt plus CpTI plants was 50 (from 14 Orders and 37 Families), comprised of 27 pest and 23 beneficial species. How about on non-transgenic control?????. The same pattern has also been observed in transgenic Bt cotton (Xia *et al.* 2001).

5.2 Effects on characteristic indices

As shown in Table 7, the dominance indices of three major sucking pests (*A. gossypii*, *T. cinnabarinus* and *T. tabaci*) and three major predators (*E. graminicolum*, *O. minutus* and *C. septempunctata*) on the transgenic Bt plus CpTI cotton were slightly increased, compared with the non-transgenic control. These results were consistent with those observed from the field-population dynamics of those pests (Fig 1and 2) and predatory species (Figs 3 and 4). However, the dominance indices of *H. armigera* and its larval parasitoids were significantly decreased compared with the non-transgenic control (Table 7). Interestingly, the dominance index of *A. gossypii* parasitoid complex on the transgenic plants was decreased by over 30 % compared with the non-transgenic control in laboratory experiments, which was not coupled with the increased abundance of *A. gossypii* on the transgenic plants (Fig.1). This may be an indication that some metabolic changes in transgenic cotton plants (e.g. changes in content of some secondary compounds) could exert certain adverse effects on *A. gossypii* parasitoids attacking the transgenic Bt plus CpTI cotton-fed hosts.

Three characteristic indices (diversity, evenness and dominance concentration) are considered important indicators of stability for an arthropod community. In general, an arthropod community is more stable when it is higher in diversity and evenness but lower in dominance concentration and complexity (Cui, 1998; Xia *et al.* 1995a, 1998). The index of diversity and evenness on the transgenic Bt plus CpTI plants was decreased compared with the non-transgenic control (Table 7). Presumably, the arthropod community on transgenic plants might be more stable than that on the non-transgenic control.

6. General Discussions and Future Research

6.1 Insect Resistance

Transgenic Bt plus CpTI cottons demonstrated a high efficacy to control *H.armigera* in laboratory, and in field experiments, though their resistance levels varied with the crop stage and plant structure. The spatiotemporal dynamics of insect resistance in the transgenic cotton could be mainly attributed to the variation in Bt expression with crop age (Sachs *et al*. 1998) and among plant structures (Benedict *et al*.1996; Sims *et al*. 1996). Spatiotemporal changes in Bt expression might be caused by genetic and environmental factors such as site of insertion, gene construct, background genotype, epitasis, somaclonal mutations, metabolism associated with plant growth and reproduction, temperature, nitrogen and soil water (Benedict *et al.* 1993, 1996; Sachs *et al.* 1998). The spatiotemporal variation in Bt expression of current transgenic Bt plus CpTI cottons would provide an ideal environment for selection of endotoxin resistance by insects, thus challenging the high-dosage strategy for endotoxin resistance management (Fitt *et al*. 1994; Forrester, 1994; Xia *et al*.1999). Variation in Bt expression could also pose a question on how to control *H. armigera* at the late crop stage if population numbers would be above the recommended action threshold for conventional cultivars.

Two main mechanisms are involved in the resistance of transgenic Bt cottons to target pests: (1) by poisoning and killing the target pests at the specific stage (larvae), and (2) by reducing reproductive potential of the infested pests (Benedict *et al*. 1992, 1993; Dong *et al.* 1997a ; Xia *et al.* 1999). Decreased reproductive potential of Bt plus CpTI cotton-infested *H. armigera* would increase, to some extent, the effectiveness of control, the significance of which needs to be further examined and understood.

6.2 Ecological Impacts

There are a number of potential ecological impacts of transgenic Bt plus CpTI cottons in cotton production systems, among which, three should be taken in to consideration from the entomological point of view.

Impact on pest species. Transgenic Bt plus CPTI cottons prove effective for controlling a variety of lepidopterous pests, *Helicovepa* spp., *H. virescens*, *P. gossypiella* and *A. ipsilon*. All these species may pose some risk of evolving resistance to the Bt endotoxin (Fitt *et al*. 1994 ; Forrester, 1994; Roush, 1994). It has been observed that some non-target sucking pests, such as *A. gossypii,* and *T. cinnabarinus* are more abundant on transgenic Bt plus CpTI cottons than on the non-transgenic cultivars. Reduced chemical use for controlling the main target bollworms on transgenic Bt plus CpTI cottons may favor some minor pests such as *Empoasca biguttula* Shiraki, *Creontiades dilutus* Stal, *Campylomma livida* Reuter and *Lygus luconum* Meyer-dur (Cui, 1998; Cui and Xia, 1998; Fitt *et al*.1994). These sucking insects may, in turn, be so important that pesticides may become a critical management option, thereby devaluing the gains from the transgenic expression system. Population dynamics of major non-target sucking pests should be well understood for effective integrated pest management in transgenic Bt plus CpTI cottons.

Impact on natural enemies. Potential impact of transgenic cottons on beneficial arthropods is derived from removal of lepidopterous eggs, larvae and pupae as food source for predators or as hosts for parasitoids. The significance of this impact depends largely on the importance of the lepidopterous life stage on cotton in maintaining local beneficial populations, and also on their feeding ecology. Clearly, transgenic Bt plus CpTI cottons impose a greater impact on the specialized parasitoids than on the generalist predators. Predators closely associated with lepidopterous pests (e.g. *P. japonica*) suffer a greater adverse effect compared with the more generalist ones (see also Helbeck *et al.* 1998a, b). *Trichogramma* spp., the important bollworm egg parasitoids, may also be influenced due to the deterioration of eggs laid by Bt plus CpTI cotton-infested adults. The effect of transgenic Bt plus CpTI cottons on the bionomics and feeding ecology of these important beneficial species should be further investigated.

Impact on endotoxin resistance. The development of insect resistance to Bt proteins is, currently, of the greatest concern to the scientists and public (Benedict *et al.* 1993; Fitt *et al.* 1994; Forrester, 1994; Kennedy and Whalon, 1995. With the ecology of *H. armigera* and its historical development of insecticide resistance taken into consideration, it seems that this insect is likely to develop resistance to transgenic Bt cotton, as there is a persistent expression of Bt proteins in them. The persistent Bt expression in host plants may lead to the potential for continuous selection for resistance. Even the transgenic Bt plus CpTI cotton possesses two genes, its risk for development of endotoxin resistance should be carefully evaluated.

6.3 Management prospects

The development, evaluation and implementation of resistance management strategies for transgenic Bt plus CpTI cotton may be a major factor for their commercialization in China cotton region because of the lesson from *H. armigera* outbreaks, mainly attributed to pyrethroid resistance (Xia, 1993, 1994). Several resistance management strategies for transgenic Bt cottons have been developed and evaluated, such as a mixture of transgenic with non-transgenic seeds, cultivation of refugia, and high dose exposure (Mcgaughey and Whalon, 1992; Roush, 1994; Xia *et al.* 2003). The seed mixture would cause severe damage to the crops as old bollworm larvae could disperse from non-transgenic to the transgenic plants (Dong *et al*.1998b; Xia *et al*. 1995b). The refuge strategy would be not necessary (provided that there is no simultaneous cultivation of other transgenic Bt crops), as there exist diversified multi-cropping patterns for bollworm hosts, and also, bollworm adults are highly mobile (Xia, 1994; Zhao *et al*. 1998b). The high dosage strategy appears to be more applicable and feasible, though it still faces some challenges, such as a lower Bt-expression in flowers of the current transgenic Bt lines, and later in the crop season. Thus, more efforts should be made on time-specific or tissue-specific expression of Bt endotoxins in transgenic plants, and to pyramid, in the same plants, the Bt endotoxin with other foreign insecticidal genes or with the native insect resistance traits (e.g. nectariless, okra leaf and high terpenoid content) (Benedict *et al*.1993; Cui and Guo, 1995; Sachs *et al*. 1996, 1998; Wilson *et al.*1992; Xia, 1996).

It should be remembered that management of Bt-endotoxin resistance is only one component in integrated cotton pest management system. As current transgenic Bt plus CPTI cotton prove effective only for controlling some lepidopterous pests, there is a need for developing a more comprehensive transgenic cotton-based system for integrated pest management on cotton. For these reasons, the seasonal pattern of arthropod communities and population ecology of some key insects at different trophic levels on transgenic cottons should be well understood. Our findings from these preliminary studies showed evidence of alterations in structures of arthropod communities and in population dynamics of some important insect species at different trophic levels on transgenic Bt plus CpTI cotton. Because of such changes, some conventional pest control tactics should be adopted accordingly. Thus, application of selective pesticides should be highlighted to optimize natural controls. Economic thresholds of important pests (e.g. *A. gossypii, T. cinnabari*, and *H. armigera* at late season) may have to be re-determined due to the changes in pest status and beneficial insect abundance. Further research on all these aspects, accompanied with a better understanding of the ecological impacts of transgenic Bt cottons on arthropod communities at each of three trophic levels, would greatly assist in scientifically utilizing modern transgenic cotton crops.

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Table 1 Effect of transgenic Bt plus CpTI cotton on mortality of cotton bollworm larvae

Month	Varieties	Mortality of cotton bollworm larvae (%)					
		Leaf	Square	Petal	Flower	Bract	Boll
Jun.	Bt+CpTI	98.2±1.53a	98.9±1.85a	---	---	98.5±3.41a	---
	Non-Bt						
Jul.	Bt+CpTI	64.3±2.12a	94.1±3.33a	92.9±0.98a	70.3±7.26a	94.3±4.12a	94.3±4.12a
	Non-Bt						
Aug.	Bt+CpTI	65.7±12.76a	87.6±14.07a	96.9±0.69a	87.8±12.16a	100±0.0a	88.4±7.24a
	Non-Bt						
Sept.	Bt+CpTI	35.2±14.72a	97.1±0.46a	97.0±2.63a	81.5±2.98a	85.6±1.96a	86.0±3.00a
	Non-Bt						

The same letter indicates the difference is not significant, and the different letter indicates the difference is significant ($p<0.05$ or $p<0.01$).

Table 2 Efficacy of transgenic Bt plus CpTI cotton to different instar larvae of cotton bollworm in the 6th day

Structures	Cultivars	Mortality of different instar larvae of cotton bollworm (%)					
		1st	2sd	3rd	4th	5th	6th
Leaf	Bt+CpTI	98.7±1.2A	98.9±1.9A	96.7±3.4A	86.8±10.0A	50.0±8.8A	21.1±5.10A
	Non-Bt	28.0±5.3B	30.0±3.3B	7.8±3.8B	15.5±3.8B	5.5±3.9B	5.5±3.8B
Square	Bt+CpTI	99.3±1.2A	84.4±7.6A	85.6±5.1aA	55.6±8.4A	35.6±5.1A	35.6±15.4A
	Non Bt	44.0±5.3B	6.7±3.4B	28.4±8.8B	18.9±11.7B	4.4±5.1B	16.7±6.6B
Flower	Bt+CpTI	91.1±2.0aA	97.8±1.9aA	81.1±15.8aA	81.1±1.9A	30.0±7.6A	17.8±6.9A
	Non Bt	40.0±20.2B	39.4±1.9B	23.3±10.0B	20.0±8.8C	5.6±5.1B	5.6±1.9B
petal	Bt+CpTI	96.1±1.0aA	100±0.0A	93.3±3.4aA	54.4±11.7A	36.7±9.0A	21.2±8.4A
	Non Bt	45.0±5.7B	38.3±8.6B	21.1±5.1B	23.3±6.7B	7.8±5.1B	0.0±0.0B
Bract	Bt+CpTI	100±0.0A	96.7±3.4A	87.8±7.7A	53.3±4.4A	40.0±6.7A	22.2±10.2A
	Non Bt	28.9±3.5C	18.9±10.2C	17.8±3.8B	15.5±2.5B	17.8±1.9B	4.5±3.9B
Boll	Bt+CpTI	96.7±1.6A	75.0±6.7aA	68.9±1.9A	48.9±1.9aA	26.7±8.8a	16.7±3.4a
	Non Bt	67.3±4.1B	25.5±6.9B	27.8±5.1B	13.3±6.7B	4.5±3.8b	1.1±1.9b

The same letter indicates the difference is not significant, and the different letter indicates the difference is significant ($p<0.05$ or $p<0.01$).

Table 3 Effect of transgenic Bt plus CpTI cotton on the development of 6th instar larvae of cotton bollworm

Items	Treatments	Leaf	Square	Flower	Petal	Bract	Boll
Weight of larvae (mg)	Bt+CpTI	261.5±72.9a	181.1±21.4a	252.0±19.7a	244.8±10.8a	179.5±14.0a	238.2±9.6a
	Non-Bt	373.5±16.2c	290.1±32.5c	319.7±23.1c	312.7±15.2c	284.5±6.4c	338.9±45.6c
Larval duration (d)	Bt+CpTI	3.2±0.2a	4.5±0.2a	4.0±0.2a	4.2±0.8a	5.1±0.3a	4.0±0.1a
	Non-Bt	2.1±0.1b	3.6±0.5b	3.3±0.6b	4.2±0.3a	5.1±0.1a	3.3±0.6b
Pupated rate (%)	Bt+CpTI	25.6±12.6aA	44.4±2.0aA	53.3±3.4aA	55.5±6.9A	25.5±15.4A	58.9±13.4A
	Non-Bt	87.2±6.1B	82.2±8.4B	77.8±5.1B	83.3±3.2C	75.6±2.0bB	90.0±3.4B
Weight of pupae (mg)	Bt+CpTI	144.7±15.3a	168.5±20.3a	203.9±10.2a	181.6±6.0a	131.4±1.3a	207.8±17.0a
	Non-Bt	220.4±27.6c	242.3±34.0c	262.0±8.5c	205.7±3.0c	211.8±12.8c	257.3±7.1c
Pupae duration (d)	Bt+CpTI	11.5±0.7a	10.0±1.0a	9.7±0.6a	9.4±0.5a	9.8±1.1a	10.3±1.2a
	Non;Bt	9.0±0.0b	10.2±0.3a	10.0±0.0a	9.0±0.0a	10.0±0.0a	9.7±1.2a
Molting rate (%)	Bt+CpTI	25.6±12.6A	36.7±3.4A	27.8±8.4aA	32.3±1.9A	5.6±5.1A	45.5±10.7a
	Non;Bt	82.2±6.9B	72.2±3.9B	62.9±8.4B	78.9±2.0C	63.3±11.6B	61.1±5.1a
Lifespan of adult(d)	Bt+CpTI	5.3±0.6a	6.0±0.9a	9.8±0.3a	8.0±1.0a	9.5±1.4a	10.3±0.3a
	Non;Bt	8.8±1.0b	7.8±0.8a	10.0±0.0a	10.0±1.0a	8.7±1.2a	10.3±0.6a

The same letter indicates the difference is not significant, and the different letter indicates the difference is significant ($p<0.05$ or $p<0.01$).

Table 4 Survival and growth of *A. ipsilon* fed transgenic Bt plus CpTI cotton leaves in laboratory

Items	Bt+CpTI	Non-Bt
Larval weight (g) at day 6	0.0031±0.0004bA	0.0040±0.0010cA
% larval survival to pupation	30.0±3.3aA	85.0±5.0bA
Larval duration (d)	30.7±2.08aAB	28.4±1.115bB
Pupa weight (g) at day 6	0.3224±0.0454aA	0.4040±0.0446bA
% pupal survival to adults	25.56±5.09aA	75.0±0.00bB
Pupal duration (d)	13.1±0.31bA	11.3±0.10aB

The same letter indicates the difference is not significant, and the different letter indicates the difference is significant ($p<0.05$ or $p<0.01$).

Table 5. Maximum Predation rate (N_a， d^{-1}), search rate (a, d d^{-1}) and handling time (T_h, h) of four major predators preying on *H. armigera* young larvae, fed transgenic Bt plus CpTI and non-transgeic cotton leaves in laboratory, based on type Ⅱ functional response of Holling (1959)

Predator	Treatment	Na	a	Th
p.japonica	Bt+CpTI	125.1	1.1213	0.0080
	Non- Bt	88.9	1.1774	0..113
C.septempunctata	Bt+CpTI	243.0	0.7263	0.0041
	Non- Bt	97.1	1.1419	0.0103
O.minutus	Bt+CpTI	84.9	0.4994	0.0118
	Non- Bt	69.0	0.4421	0.0143
E.graminicolum	Bt+CpTI	49.2	0.7309	0.2030
	Non- Bt	35.9	1.3740	0.2785

The same letter indicates the difference is not significant, and the different letter indicates the difference is significant ($p<0.05$ or $p<0.01$).

Table 6. Survival and growth of *Microplitis* sp. and *C. chlorideae* parasitizing *H. armigera* young larvae (n=50), fed transgenic Bt plus CpTI and non-transgenic cotton leaves in laboratory

Parameter	Treatment	C.chlorideae	Microplitis sp.
%larvae parasitized	Bt+CpTI	43.3 aA	21.6 aAB
	Non- Bt	80.6Ac	32.6 aAC
Cocoon wt at day 3(g)	Bt+CpTI	0.0067 aA	0.0038aA
	Non- Bt	0.0087cB	0.0051 cC
%adult emergence	Bt+CpTI	73.6 aA	47.7aA
	Non- Bt	100cC	100 cC
Adult wt at emergence(g)	Bt+CpTI	0.0008 aA	0.0006aA
	Non- Bt	0.0009bA	0.0007 bA

The same letter indicates the difference is not significant, and the different letter indicates the difference is significant ($p<0.05$ or $p<0.01$).

Table 7 Comparison of characteristic indexes of arthropod communities on transgenic Bt plus CpTI and non-transgenic cotton plants in an unsprayed field

Parameter	Bt+CpTI	Non- Bt
Dominance index		
A.gossypii	0.8817	0.8067
T.tabaci	0.0233	-
H.armigera	-	0.0137
E.graminicolum	0.3559	0.2150
C.septempunctata	0.0717	0.0811
P.japonica	0.2629	0.1923
O.minutus	0.0186	0.571
Diversity index	1.2157±0.1933aA	1.1185±0.1533 abA
Evenness index	0.3109±0.0538 aA	0.2857±0.0335 aA
Dominance concentration index	0.5731±0.073 abA	0.6183±0.0536 abA

The same letter indicates the difference is not significant, and the different letter indicates the difference is significant ($p<0.05$ or $p<0.01$).

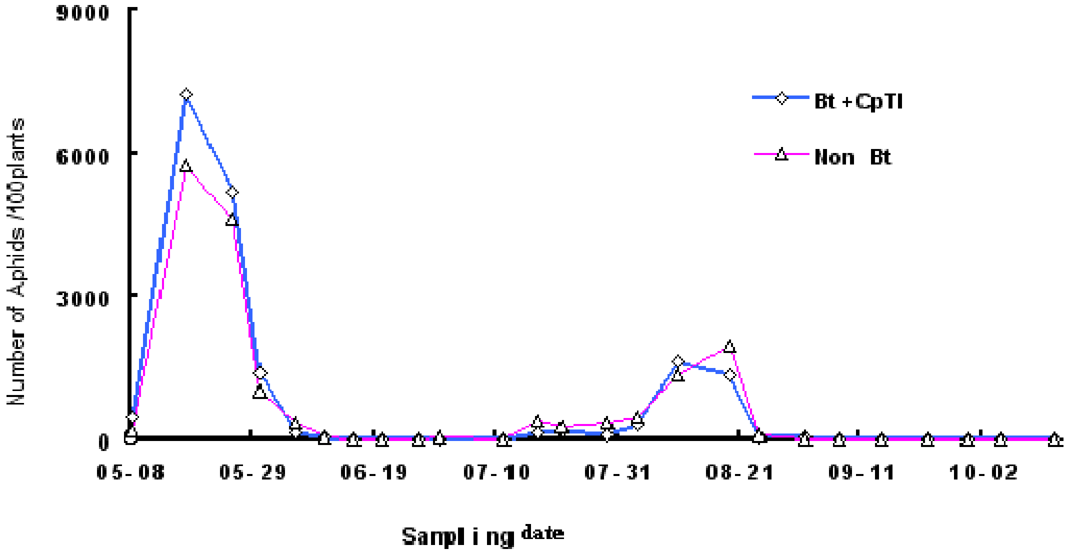

Fig.1. Population dynamics of *A. gossypii* in transgenic Bt plus CpTI cotton

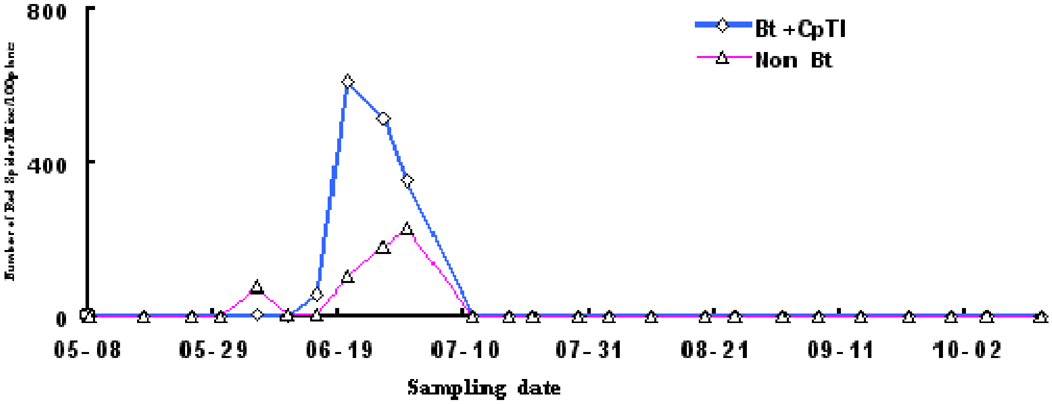

Fig 2 Population dynamics of *T. cinnabarinus* in Bt plus CpTI cotton

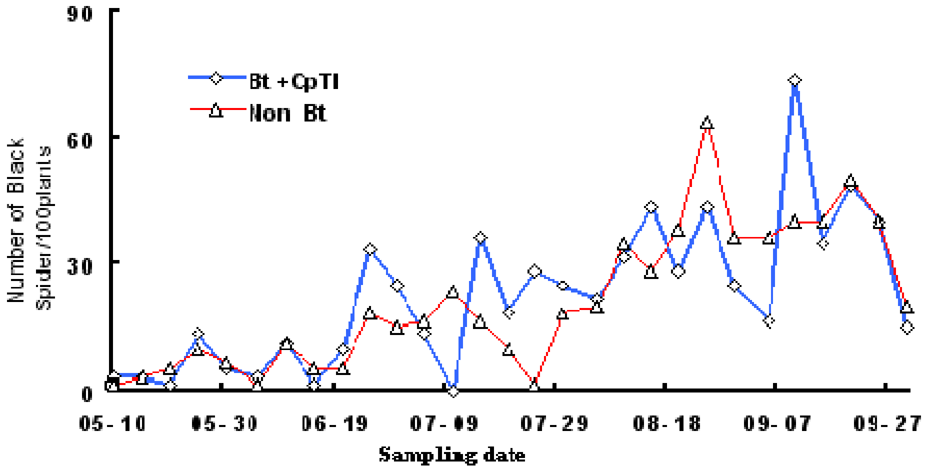

Fig.3 Population dynamics of *E. gramicicolum* in transgenic Bt plus CpTI cotton

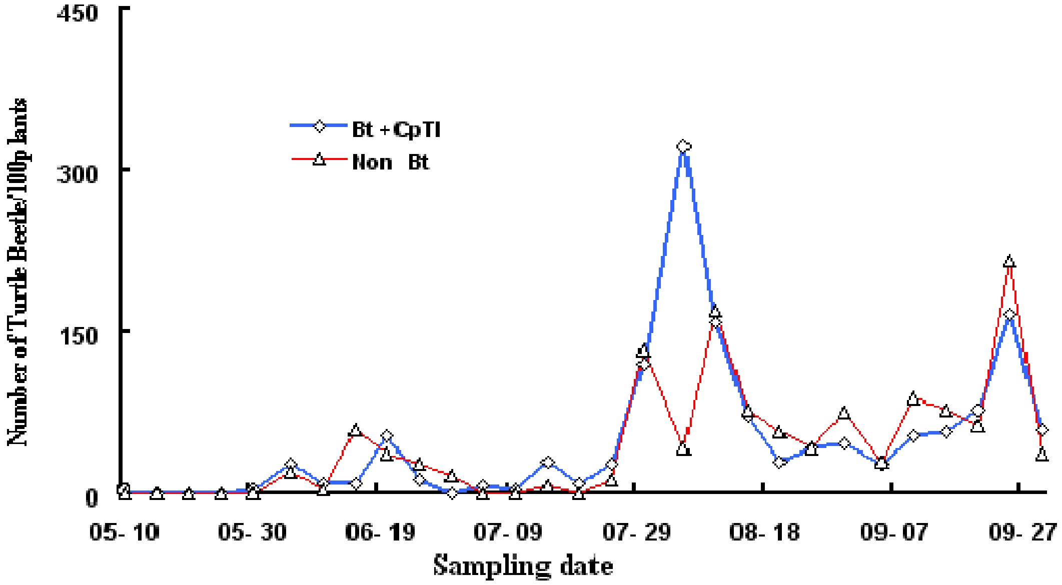

Fig.4 Population dynamics of *P. japonica*) in transgenic Bt plus CpTI cotton

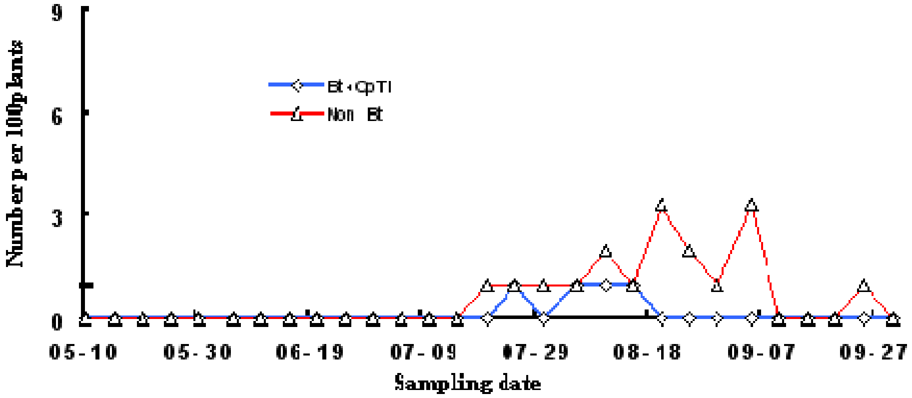

Fig. 5. Population dynamics of *Microplitis* sp. n transgenic Bt plus CpTI cotton