A SUMMARY OF RESEARCH ON
THE ENVIROMENTAL IMPACTA OF

BT COTTON IN CHINA

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List of Acronyms & Abbreviations

Bt	*Bacillus thuringiensis* Berliner
Bt cotton	*Bt* transgenic cotton
CK	Check (control treatment)
CpTI	Cowpea trypsin inhibitor
CAAS	Chinese Academy of Agricultural Sciences
GMO	Genetically modified organism
IPM	Integrated pest management
LMO	Living modified organism
IC_{50}	Inhibitory concentration 50 – concentration that produces 50% inhibition of larval development
LC_{50}	Lethal concentration 50 – concentration that kills 50% of individuals

EXECUTIVE SUMMARY

Currently, *Bt* (*Bacillus thuringiensis* Berliner) transgenic cotton is the main GMO crop variety in large-scale commercial production in China. Due to the introduction and popularization of Monsanto's *Bt* (transgenic) cotton since 1997, *Bt* cotton plantings have had a very fast growth in area. In 2000, *Bt* cotton was grown on up to 1 million hectares, accounting for 30% of cotton production in China. It is estimated that the area planted to *Bt* cotton has increased to 1.5 million hectares in 2001, on 35% of the total cotton area. Monsanto's *Bt* cotton accounts for approximately two thirds of the *Bt* cotton grown, while the several domestically developed *Bt* cotton varieties account for the remaining one third.

Research conducted during the past few years at four domestic academic institutions shows that *Bt* cotton is effective in controlling the primary pest of cotton – bollworm (*Helicoverpa armigera* H✓bner), especially in seedling stage of cotton. However, laboratory experiments and field research also demonstrate that there are adverse environmental impacts associated with the cultivation of *Bt* cotton. These impacts are summarized below.

1. Although in the Chinese studies there are no significant impacts on predatory natural enemies associated with *Bt* cotton, there are associated adverse impacts on parasitic natural enemies of cotton bollworm. In *Bt* cotton fields, researchers have shown a decrease in the ratios of parasitization and eclosion and reduction in the weights of cocoon and adult. Consequently, the populations of parasitic natural enemies in *Bt* cotton fields are significantly reduced.

2. *Bt* cotton is not effective in controlling many secondary pests, especially sucking pests. Field experiments showed that the populations of secondary pests such as cotton aphids, cotton spider mites, thrips, lygus bugs, cotton whitefly, cotton leaf hopper and beet armyworm increased in *Bt* cotton fields after the target pest – bollworm – had been controlled. Some pests replaced bollworm as primary pests and damaged cotton growth.

3. The diversity indices of the insect community, the pest sub-community and the pest-natural enemies sub-community, as well as the evenness index of *Bt* cotton fields are all lower than those in conventional cotton fields. However, the pest dominant concentration in *Bt* cotton fields is higher than in the conventional cotton fields. Therefore, the stabilities of insect community, pest sub-community and pest-natural enemies sub-community in *Bt* cotton fields may be less than those in conventional cotton fields, and the possibility of outbreaks of certain pests in *Bt* cotton is much higher.

4. Both laboratory tests and field monitoring have verified that cotton bollworm can develop resistance to *Bt* cotton. Laboratory tests for selection of *Bt*-resistant bollworm indicated that susceptibility of bollworm to *Bt* cotton fell to 30% after 17 generations under continuous selection with a diet of *Bt* cotton leaves. The resistance index of the bollworm increased 1000 times when the selection was continued to the 40th generation. Based on these results, the scientists concluded that *Bt* cotton would probably lose its resistance to bollworm in fields after the *Bt* cotton has been planted for 8-10 years continuously.

5. *Bt* cotton demonstrates excellent resistance to the second generation bollworm and chemical control is not generally needed for the seedling period of *Bt* cotton. However, the resistance of *Bt* cotton to bollworm decreases over time, and control is not complete in the third and fourth generations. In fact, farmers must use chemicals 2-3 times to control bollworm, particularly from mid- July to the end of August.

6. Development of resistance of bollworm to *Bt* cotton has been commonly recognized in China, but there are not yet effective measures to postpone resistance development or to resolve the resistance problem. A high-dose of the *Bt* toxin protein is considered difficult to obtain, and the refuge mechanism is not easily implemented. In addition, the high-dose assumption and refuge design have theoretical shortcomings.

INTRODUCTION

1. *Bt* Cotton Expansion in China

Bt cotton plantings in China have expanded quickly and the percentage of cotton planted in *Bt* varieties area has seen increasing growth in China during the past several years. *Bt* cotton area was over 1 million hectares in 2000, about 30% of the whole cotton area in China. It is estimated that *Bt* cotton will account for up to 1.5 million or so hectares in 2001, accounting for about 35% of whole cotton area in the country (Table 1).

Table 1 *Bt* cotton Plantation in China (1996~2001)

Year	Total *Bt* cotton (ha)	Total cotton area (ha)	*Bt* cotton (%)
1996	16 667	4 720 000	0.35
1997	34 000	4 490 000	0.76
1998	228 000	3 868 667	5.89
1999	578 000	3 169 333	18.24
2000	1 076 000	3 600 000	29.89
2001	*Est.1.4 – 1.8 mill.*	*Est.4 733 333*	*Est. 29.6-38.0*

Data source: Cui Jinjie, Cotton Research Institute of CAAS.

2. Monsanto *Bt* Cotton Has a Large Market in China

Monsanto received a permit in 1997 for commercial production of *Bt* cotton. As the company has competent capacity in marketing, Monsanto's *Bt* cotton has come to occupy more than 65% of the *Bt* cotton area in China (Table 2). However, it is noted that domestically developed *Bt* cotton accounts for one-third of *Bt* cotton seed market and this percentage should increase over the next few years. The main domestic *Bt* cotton varieties include the GK Series (such as GK-2, GK-12) and stacked varieties with *Bt* plus cowpea trypsin inhibitor – CpTI (such as Shiyuan 321) developed by the Biotechnology Center of the Chinese Academy of Agricultural Sciences (CAAS), the Zhong-Mei Series (such as Zhong-29 and Zhong-30) developed by the Cotton Research Institute of CAAS and others.

Table 2 Monsanto *Bt* Cotton in China (1996~2001)

Year	Monsanto *Bt* cotton (ha)	Domestic *Bt* cotton (ha)	Total *Bt* cotton (ha)	Monsanto *Bt* cotton (%)
1996	0	16 667	16 667	0
1997	12 667	21 333	34 000	37.3
1998	182 667	45 333	228 000	80.1
1999	393 333	184 667	578 000	68.1
2000	709 333	366 667	1 076 000	65.9
2001	*Est. 1.0-1.3 mill.*	*Est. 0.4-0.5 mill.*	*Est.1.4-1.8 mill.*	*71-72*

Data source: Cui Jinjie, Cotton Research Institute of CAAS.

3. *Bt* Cotton Distribution in China

Bt cotton cultivation has been widespread in China and popularized in more than 10 provinces. Currently, *Bt* cotton is mainly planted in Yellow River Valley of North China, including Shandong, Henan, Shanxi and Hebei provinces. *Bt* cotton acreage is expanding quickly in the Yangtse River Valley of South China, predominantly in Hubei, Hunan, Anhui and Jiangsu provinces. In 2000, about 75% *Bt* cotton was planted in the Yellow River Valley, 24% in the Yangtse River Valley, and 1% in the Xinjiang Autonomous Region located in Northwest China. (Piao, 2001, FAO meeting) It is estimated that *Bt* cotton area will be greatly increased in Yangtse River Valley provinces in 2001.

4. Research on *Bt* Cotton's Environmental Impacts

Since beginning of *Bt* cotton cultivation in China in 1997, some Chinese scientists have been paying great attention to potential adverse effects of *Bt* cotton on biodiversity and the environment, especially on non-target organisms and *Bt*-resistance of cotton bollworm. Many studies on the issues have been conducted during the past five years. The main researchers and institutions are listed as follows:

1. Prof. Wu Kongming and others, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS), Beijing. Besides laboratory tests, his field studies are based in Xinxiang, Henan Province and Langfang, Hebei Province from 1997. Several other scientists at the Institute are also engaged in *Bt* cotton assessment research.

2. Prof. Xia Jingyuan, Dr Cui Jinjie and others, Cotton Research Institute of CAAS, located in Anyang, Henan Province. Since 1995, they have been focusing on *Bt* cotton research. Their field studies are based at the Experimental Farm of the Institute in Anyang, Henan Province.

3. Prof. Zhang Qingwen and his students, Department of Plant Protection, China Agricultural University, Beijing. Their field studies have been based in Hubei Province (Yangtse River Valley) and Xinjiang Autonomous Region since 1998.

4. Prof. Shen Jinliang, Department of Plant Protection, Nanjing Agricultural University, Located in Nanjing, Jiangsu Province. His studies are based in both laboratory and fields mainly in Jiangsu Province (Yangtse River Valley).

Currently the Ministry of Science and Technology of Chinese Central Government is paying great attention to the environmental assessment of GMOs. Not only was GMO safety assessment listed in the existing National Key Hi-tech "863 Plan", but also the safety research has been put in the new National Key "973 Plan". Additionally, a special research fund was established for *Bt* cotton environmental assessment. An increasing number of scientists and institutes are becoming involved in *Bt* cotton research.

The author interviewed scientists from the institutes mentioned above during 2001 to assess the state of research on environmental impacts of *Bt* cotton in China. Based on the data collected in these interviews, this report summarizes the results of these studies and documents adverse impacts of *Bt* cotton on the environment and biodiversity.

BT COTTON'S IMPACTS ON NON-TARGET ORGANISMS

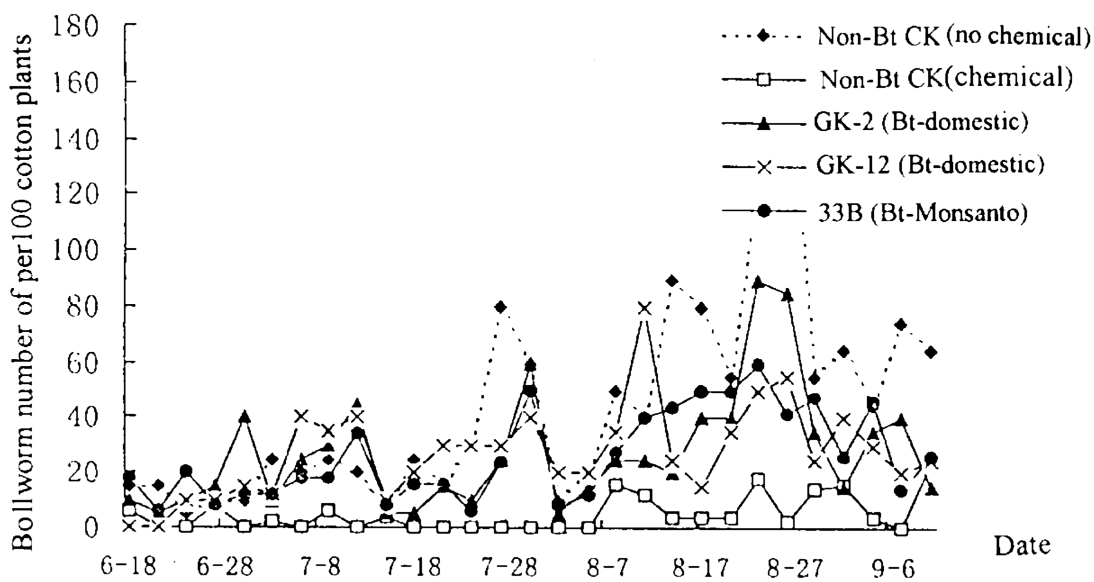
In China, the most important pest for cotton production is the cotton bollworm (*Helicoverpa armigera* H✓♌ner), especially in North China. Cotton bollworm is the target pest of transgenic *Bt* cotton; the bollworm is killed by the expressed *Bt* toxin protein inside of cotton plants. As the farm ecosystem is complicated, when the target pest is controlled by *Bt* cotton, many non-target pests and natural enemies are also influenced. For our consideration here we divide non-target organisms into pest-natural enemies and secondary pests.

1. Impacts on Natural Enemies of Bollworm

(1) Results of the studies by Prof Wu Kongming, Institute of Plant Protection, CAAS, Beijing

The tests conducted in Xinxiang, Henan Province in 1998 showed predator populations of ladybeetles, lacewings, and spiders in *Bt* cotton (Monsanto-33B, GK-2 and GK-12) to be higher than in conventional cotton (Zhong-12) grown using chemicals and much lower than in conventional cotton (Zhong-12) grown without chemicals. These results suggest that cultivation of *Bt* cotton might have negative impacts on the population dynamics of natural predators (Graph 1).

Graph 1 Population dynamics of natural enemies in different cotton fields
(Xinxiang, Henan Province, 1998)

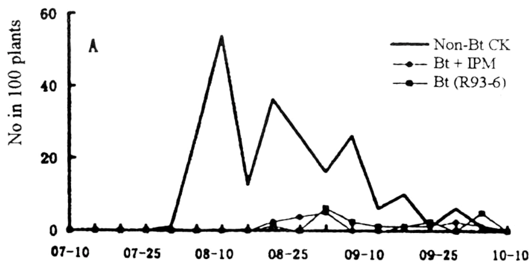


(2) Results from Dr Cui Jinjie and Prof Xia Jingyuan, Cotton Research Institute, CAAS, Anyang, Henan

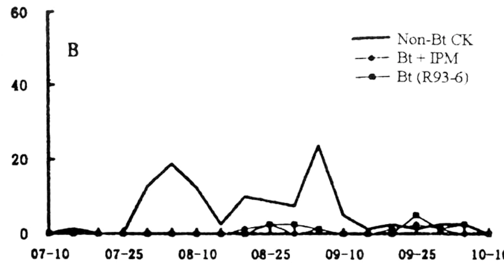
In 1997, the impacts of transgenic *Bt* cotton on the population dynamics of natural enemies were studied both in the laboratory and in the field. The results of these studies showed that the impacts of *Bt* cotton on predator population dynamics were not obvious, and the number of the predators in *Bt* fields were not significantly different compared with conventional cotton. The main natural predators studied were *Coccinella septempunctata*, *Propylaea japonica, Erigonidium graminicola, Geocoris pallidipennis, Chrysopa sp.* and *Orius mimutus.*

However, impacts of *Bt* cotton on parasitic natural enemies were demonstrated. *Microplitis sp.* and *Campoletis chlorideae* Uchida are two dominant parasitic natural enemies of the larvae of cotton bollworms. This research showed ratios of parasitization and eclosion (emergence) in *Bt* cotton decreased substantially and both the cocoon weight and adult weight were significantly reduced. The number of parasitoid populations in *Bt* cotton decreased by 88.9% (season average figure) for *Microplitis sp.* and 79.2% for *Campoletis chlorideae* Uchida compared with conventional cotton. (Graph 2) Field observation showed a significant reduction in abundance of *Microplitis sp.* and *Campoletis chlorideae* in *Bt* cotton (R93-4), and the season mean number of both parasitoid species found on the conventional non-transgenic control was 7-11 times that found on the transgenic *Bt* plants (Cui and Xia 1999). (Graph 2)

Graph 2 Population dynamics for parasitic natural enemies in *Bt* cotton
(Anyang, Henan, 1997)



A *Microplitis sp.*



B *Campoletis chlorideae* Uchida

(3) Results from Prof. Zhang Qingwen, Dept. of Plant Protection, China Agricultural University, Beijing

Studies conducted in Hubei Province (Yangtse River Valley) during 1999~2000 indicated no obvious difference between *Bt* cotton and non-*Bt* cotton (conventional cotton) for populations of most predators, with the exception that a larger population of *Propylaea japonica* Goeze was found in *Bt* cotton. (Zhang *et al.*, research report, 2001)

The studies conducted in Xinjiang in 2000 also indicated there is no significant difference in predator populations between *Bt* cotton and non-*Bt* cotton. (Wei *et al.*, 2001)

(4) Results from Prof. Shen Jinliang, Dept. of Plant Protection, Nanjing Agricultural University, Nanjing, Jiangsu Province

A. Laboratory method

The laboratory study in 2001 focused on the impacts of *Bt* cotton on the parasitic natural enemy *Microplitis mediator* Haliday, a dominant parasite which feeds specifically on the young larvae of cotton bollworm. Selected *Bt*-resistant bollworm were fed various cotton varieties: Shiyuan 321(*Bt* + CpTI), Zhong Suang Kang (*Bt* + CpTI), Xing 33B (Monsanto *Bt*) and Su 12 (local conventional cotton as a control). A *Bt*-susceptible bollworm strain fed Su 12 was also used as a control. The bollworms were then placed in containers with *Microplitis mediator*, and effects on *Microplitis mediator* observed. The results indicate that all *Microplitis mediator* parasitizing bollworms fed on *Bt* cotton and cotton with *Bt* and CpTI have decreased parasitization rate, cocoon rate, eclosion rate and cocoon weight compared with one of the controls – *Bt* susceptible bollworm fed Su 12 (non-*Bt* conventional cotton).

However, reductions in parasitization and cocooning rates of parasites on *Bt*-resistant bollworm fed Su 12 are similar to those of parasites of *Bt*-resistant bollworm feeding on *Bt* cotton (Table 3). The researchers conclude that the impacts result from two distinct, potentially interacting factors: the *Bt* toxin and the resistance of the bollworm to *Bt*. Researchers will continue their investigations in 2002 on the potential significance of each factor.

Table 3 Impacts of *Bt* cotton on growth of parasitic natural enemies (2001)

Cotton treatments	Bollworm strains	Reduction of parasitization rate (%)	Reduction of cocoon rate (%)	Reduction of eclosion rate (%)	Reduction of cocoon weight (%)
Shi Yuan 321(*Bt* + CpTI)	R	15.3	14.0	9.3	5.7
Zhong S.K.(*Bt* + CpTI)	R	55.6	59.1	69.5	20.9
Xing33B (Monsanto *Bt*)	R	49.8	51.9	68.2	17.2
Su 12 (conventional)	R	41.5	47.3	14.6	5.7
CK (Su 12)	S				

In addition, Shen used the same laboratory method above to study a predatory natural enemy, the lady beetle *Harmonia axyridis* (Pallas). Results indicate that *Bt* cotton has no impact on 2nd-4th instar larvae and adults of *Harmonia axyridis* (Pallas), but there are obvious impacts on the survival rate of 1st instar larvae. In particular, the varieties with two resistance genes (*Bt* + CpTI) have significant impacts on 1st instar larvae, with a larval mortality of 24%. (Shen, unpublished report)

B. Field tests

In 2001, Prof. Shen Jinliang conducted a field test in Funing, Jiangsu Province on parasitization rates in *Bt* cotton fields of *Campoletis chlorideae,* a parasitic natural enemy specific to larvae of cotton bollworm. His results show reduced rates of parasitization of bollworm larvae. These results also indicate that *Bt* cotton has a slight impact on the rate of parasitization of bollworm eggs (Table 4).

Table 4 Impacts of *Bt* cotton in fields on parasitic natural enemies of bollworm

Funing, Jiangsu, 2001

Treatments	Parasitization rate on the third generation of cotton bollworm (%)		Parasitization rate on the third generation of cotton bollworm (%)	
	Bollworm larvae	Bollworm egg	Bollworm larvae	Bollworm egg
33B (*Bt*-Monsanto)	0	2.63	0	1.22
Control (non-*Bt* Su Series)	4.17	3.0	6.11	1.38

2. Impacts on Secondary Pests

(1) Results from Prof. Wu Kongming, Institute of Plant Protection, CAAS

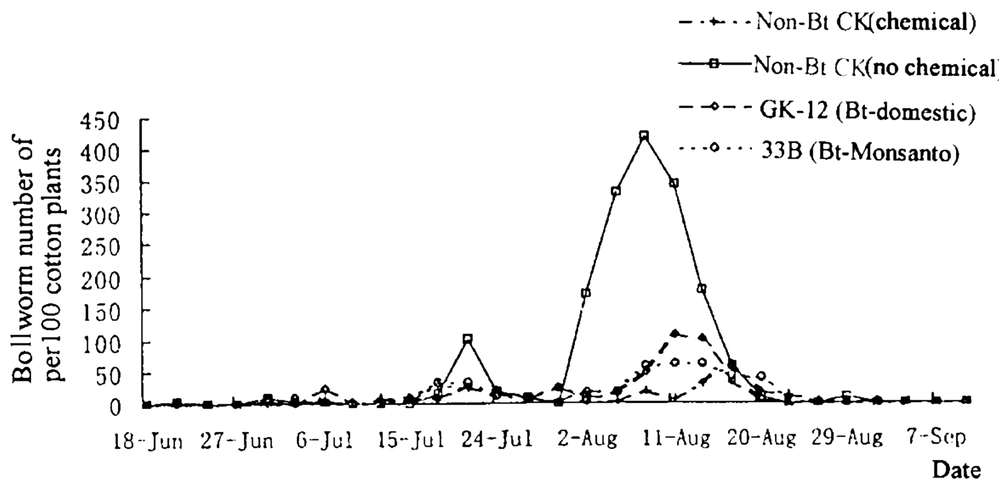
The studies were conducted in Xinxiang of Henan Province and Langfang of Hebei Province during 1997-2000. Treatments included the *Bt* cotton varieties 33B and 35B (*Bt*, Monsanto), GK 2 and GK 12 (*Bt*, domestic), Shiyuan 321 (*Bt* + CpTI, domestic), Zhong 12 (non-*Bt* control).

A. Beet armyworm (*Spodoptera exajua*)

Since 1997, when *Bt* cotton began to be planted on a large scale, beet armyworm has become an increasingly serious pest in China. Only 70% of armyworms are controlled by *Bt* cotton (Graph 3). Now, in

North China, it seems necessary to use chemicals specifically against beet armyworm when *Bt* cotton is planted.

Graph 3 Population dynamic of beet armyworms in different cotton fields
(Xinxiang, Henan, 1999)



B. Lygus bugs (*Adelphocoris saturalis, A. fasciaticolls, Lygus lucorum*, etc.)

Lygus bug populations tend to increase when the climate is humid, a trend that is exacerbated because chemical use is reduced in *Bt* cotton fields and these pests are weakly controlled by natural enemies. For example, there was a heavy rainfall in North China in 1998, which contributed to an outbreak of lygus bugs in the *Bt* cotton fields during the whole growing season. In 1999, lygus bugs were not a serious problem before July because the climate was dry, but became very serious after the middle of August (Graph 4), and led a great loss of autumn bolls. Test results from 2000 showed serious damage by lygus bugs in *Bt* cotton after Aug 12 (Wu, research report, 2001). (Table 5)

Graph 4 Population dynamic of Lygus bugs in different cotton fields
(Langfang, Hebei, 1999)

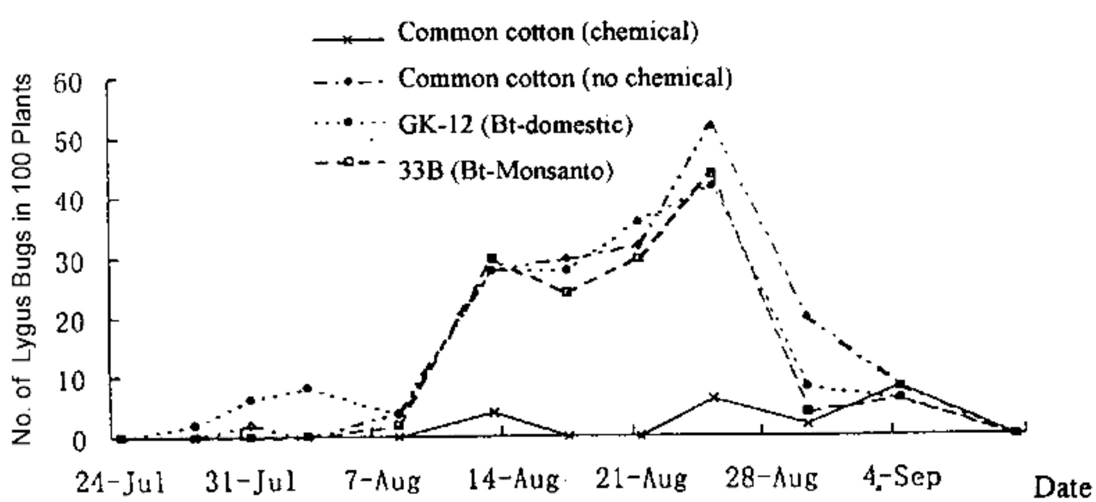


Table 5: A survey for lygus bugs in different cotton fields
(Langfang, Hebei, Aug 21, 2000)

Cotton variety	GK 12 (*Bt*)	Shiyuan 321 (*Bt* + CpTI)	Non-*Bt* CK (no chemical)	Non-*Bt* CK (chemical)
Lygus bugs pest individuals (per 100 plants	320	326	364	insignificant

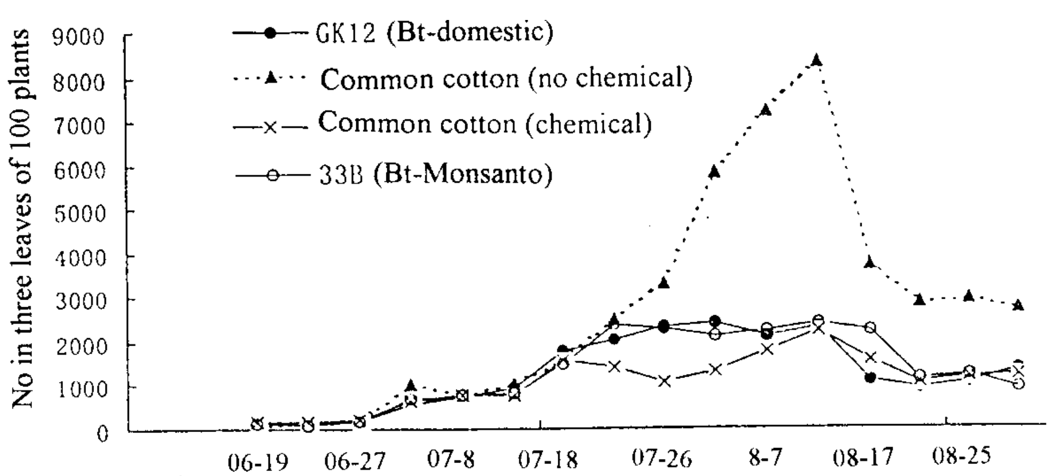
C. Cotton spider mite (*Tetranychus urticae* Koch)

Similar to lygus bugs, cotton spider mites are weakly controlled by natural enemies. Populations may increase when the climate is dry to become a serious pest in *Bt* cotton fields.

D. Tobacco whitefly (*Bemisia tabaci*)

Field test results in Langfang, Hebai Province, 2000 showed *Bt* cotton was not useful in controlling the tobacco whitefly, an expected result as the toxin is specific to lepidopteran insects. Although there was no obvious difference before July 18 for the pest population dynamics in different cotton fields, a substantial increase in whitefly populations was seen in *Bt*-cotton fields relative to non-*Bt* cotton from mid-July to mid-August (Graph 5) (Wu, research report, 2001).

Graph 5 Population dynamics of tobacco whitefly adults in different cottons
(Langfang, Hebei, 2000)



(2) Results from Dr Cui Jinjie and Prof. Xia Jingyuan, Cotton Research Institute, CAAS, Anyang, Henan Province

A field study in 1997 conducted in Anyang, Henan showed obvious adverse impacts of *Bt* cotton on the main secondary pests (Cui and Xia 1998) of cotton, including cotton aphids, spider mites, thrips, whitefly lygus bugs, and leafhoppers. Some secondary pests became primary pests in the absence of bollworm. The treatments in the field study were *Bt*-Zhong 30 (R93-6), *Bt*-Zhong 30 with integrated pest management (IPM) measures, and Zhong 16 (non-*Bt* conventional control (CK) without chemicals).

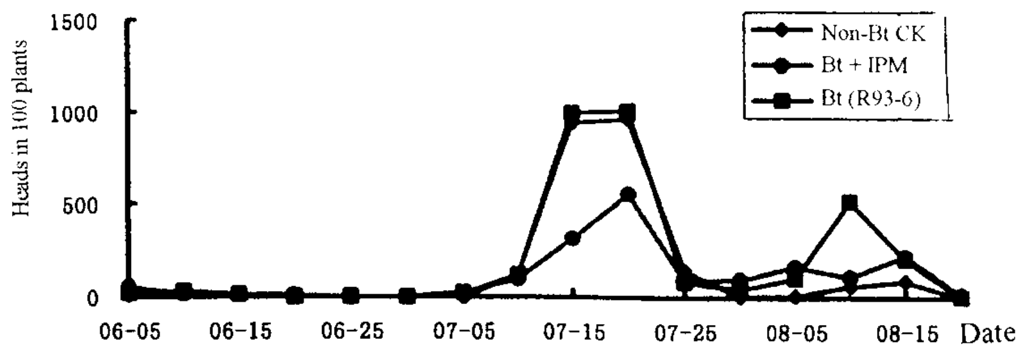
Three types of IPM measures were used in the study:

(1) use of chemicals, including monocrotophos, profenofos, phoxim, and endosulfan;
(2) biological control by biological pesticides such as nuclear polyhedrosis virus (NPV) for control of the fourth generation of cotton bollworm, and chemical control with chemicals less-damaging to natural enemies; and
(3) farming methods and cropping system measures to increase populations of natural enemies, such as by planting corn near cotton fields to serve as a refuge for natural enemies.

A. Cotton Aphids (*Aphis gossypii)*

In this study, the season mean number of cotton aphids found in the third young leaf from the top of 100 plants was 148.5 in the non-*Bt* cotton control and 197.6 in *Bt* cotton, a 33.1% increase over the course of the season compared to the non-*Bt* CK. Two population peaks occurred, one in mid-July and the other in early August. The aphid population in *Bt* cotton was much higher than that of conventional cotton, especially during the second peak. (Graph 6)

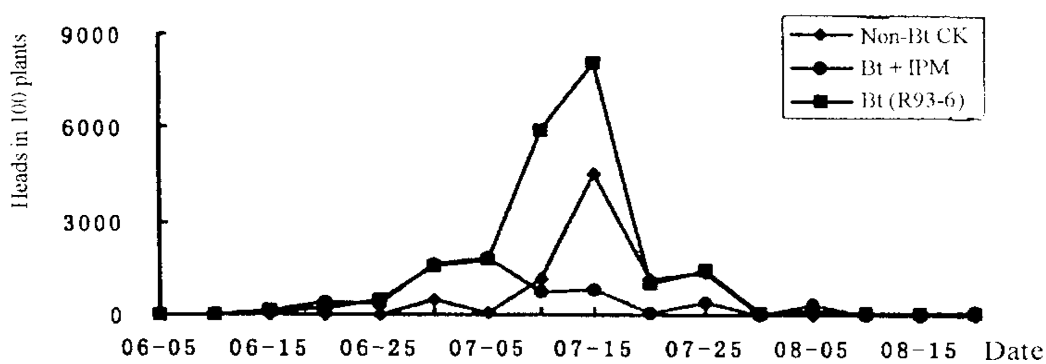
Graph 6 Population dynamics of cotton aphids
(Anyang, Henan, 1997)



B. Cotton spider mite (*Tetranychus cinnabarinus)*

In this study, the season mean number of cotton spider mites per 100 plants of *Bt* cotton (1312.4 individuals) was 138.9% of the mean number found in non-*Bt* conventional cotton (549.3 individuals). During an outbreak in mid July, *Bt* cotton fields (Graph 7) and non-*Bt* fields had populations of approximately 8000 and 4500 individuals per hundred plants respectively, while the *Bt* + IPM treatment had populations of several hundred mites per hundred plants.

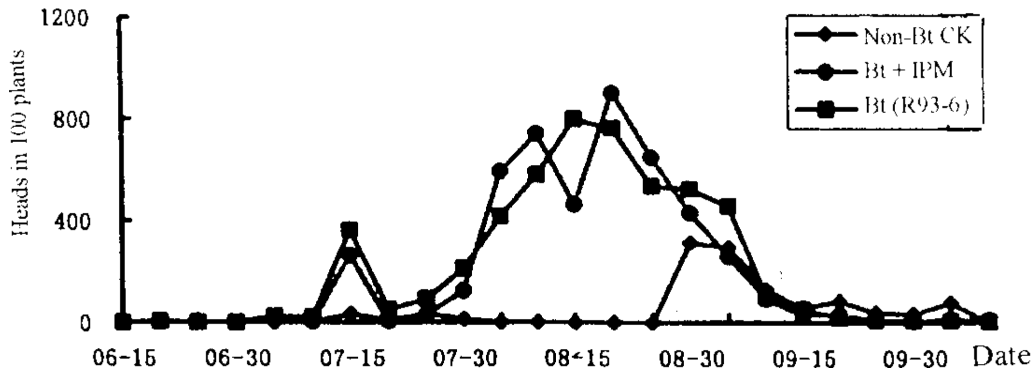
Graph 7 Population dynamics of cotton spider mite
(Anyang, Henan, 1997)



C. Lindemon onion thrips (*Thrips tabaci)*

In this study, the season mean number per 100 plants of *Thrips tabaci* in *Bt* cotton was 210.5 individuals, an increase of 346.0% compared to the non-*Bt* conventional control (non-*Bt* CK) where the season mean number was 47.2 individuals. In the peak period from the end of July to early September, the thrips population in *Bt* cotton reached over 800 individuals. The *Bt*-cotton + IPM treatment followed the same trend as the *Bt*-cotton treatment (Graph 8).

Graph 8 Population dynamics of *Thrips tabaci*
(Anyang, Henan, 1997)



D. Cotton whitefly (*Trialeurodes vaporariorum)*

The season mean number for 100 plants of *Trialeurodes vaporariorum* in *Bt* cotton was 37.2 individuals, while the number in non-*Bt* conventional control was 22.1, a 68.3% increase in *Bt* cotton fields over the non-*Bt* control (Cui and Xia 1998).

E. Lygus bugs (*Lygus luconum*)

The season mean number for 100 plants of *Lygus luconum* in *Bt* cotton was 163 individuals, a 288% increase compared with the non-*Bt* conventional control (42 individuals).

F. Cotton leaf hopper (*Empoasca biguttula)*

The season mean number of *Empoasca biguttula* was 55 individuals in *Bt* cotton and 35 individuals in non-*Bt* cotton, a 57% increase in the *Bt* cotton field compared with the non-*Bt* conventional cotton.

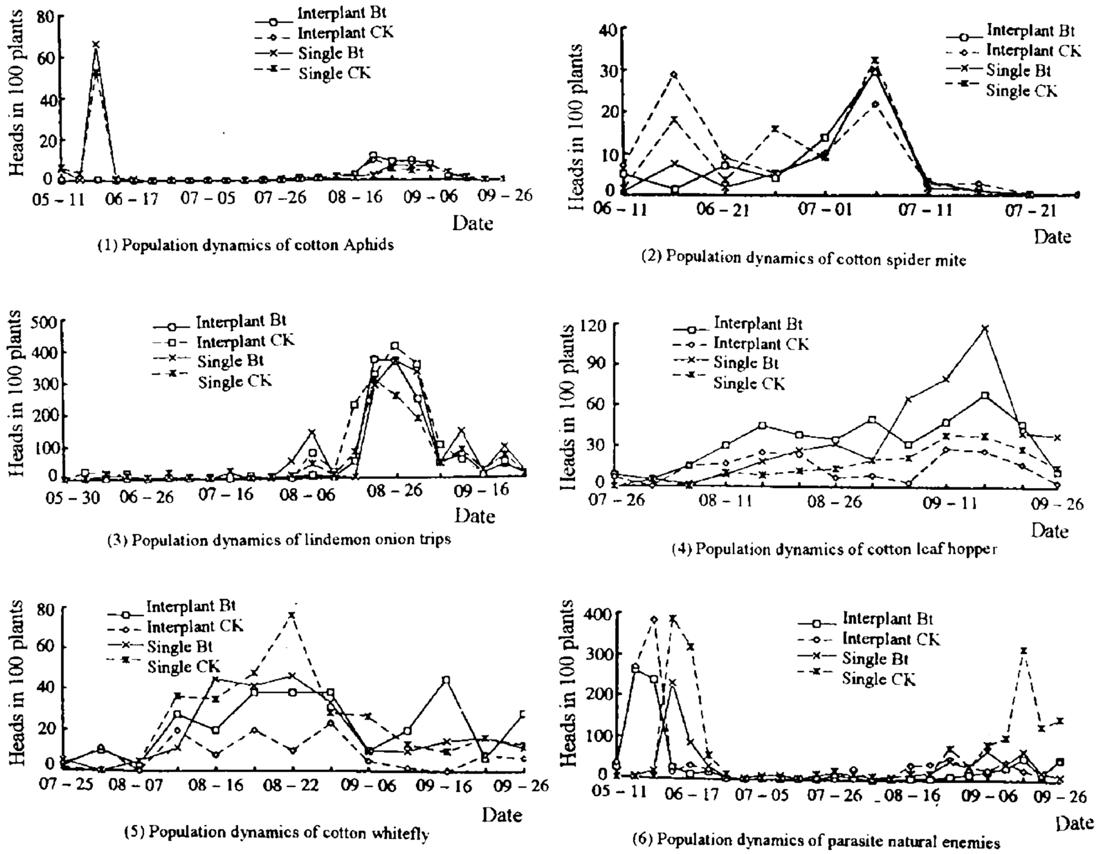
G. Impacts of *Bt* cotton on secondary pests under different cropping systems

Another study conducted by Dr. Cui and Prof. Xia in 1995 and 1996 also demonstrated impacts of *Bt* cotton on the main pests under the cultivation treatments of monocultured cotton and interplanted cotton. The common type of interplanting in China is wheat and cotton. Cotton is planted in prepared rows before the wheat is harvested. In the north area of China, cotton seeds are directly planted in the rows in May. When wheat is harvested in mid-June, the cotton seeds have already germinated and are in the seedling stage.

Under monoculture conditions, season mean numbers of seedling aphids, summer aphids and leafhoppers (*Empoasca biguttula*) in *Bt* cotton (R93-4) increased by 20.3%, 21.4% and 67.6% respectively compared to non-*Bt* conventional cotton. Season mean numbers of summer aphids, cotton spider mites, cotton whitefly (*Trialides vaporariorum*) and cotton leaf hoppers in interplanted *Bt* cotton increased by 14.6%, 75.5%, 48.2% and 58.5% respectively over interplanted non-*Bt* cotton (Graph 9) (Cui and Xia 1997).

Graph 9 Impacts of *Bt* cotton on main secondary pests of cotton

(Anyang, Henan, 1995-1996)



(3) Results from Prof. Zhang Qingwen, Dept. of Plant Protection, China Agricultural University, Beijing

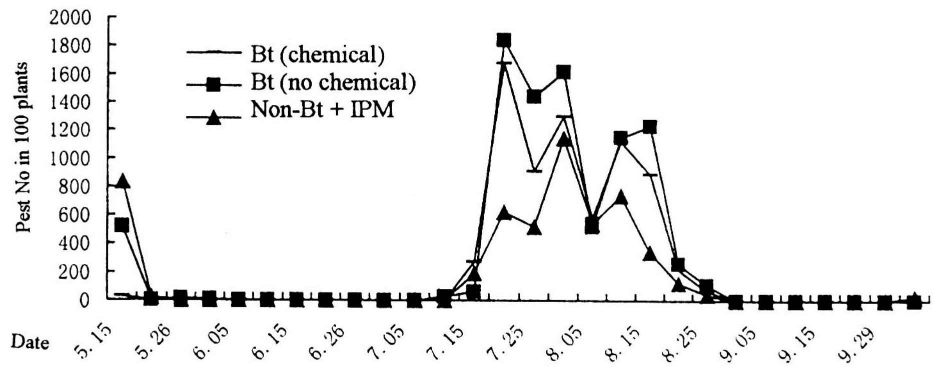
The studies were conducted in Hubei Province (Yangtse River Valley) during 1999-2000. The treatments were *Bt* cotton (GK19) without chemicals, *Bt* cotton (GK19) with chemicals and a non-*Bt* local variety (Jingzhou 5179) treated with IPM measures similar to those described on pages 11-12. The results showed that almost all the important secondary pests in two *Bt* cotton treatments had higher population levels than in non-*Bt* IPM cotton, particularly during pest outbreaks**.** The main results are detailed below (Zhang *et al.*, research report 2000).

A. Cotton aphids

The study conducted in Hubei Province showed that the number of cotton aphids (in the third young leaf from the top of 100 plants) in *Bt* cotton (GK19) reached 1840 individuals at peak in late July, much higher than in non-*Bt* conventional cotton (1140 individuals) (Graph 10). As the peak lasted to late August and this month is the crucial period for cotton growth and boll development, aphid damage could be significant.

Meanwhile, the study conducted in Xinjiang Autonomous Region showed similar results, that is, aphid numbers in 100 plants were up to 68,800 individuals at the peak in *Bt* cotton (without chemical), 35 times the number found in non-*Bt* conventional cotton – less than 2000 individuals. One interesting finding was that the aphid numbers in the treatment of *Bt* + IPM at peak population were 54,300, similar to that of *Bt*-cotton without IPM. The researchers concluded that the aphids' occurrence is strongly correlated with cotton variety (*Bt* versus non-*Bt*) rather control measures (Zhang *et al.*, research report 2000).

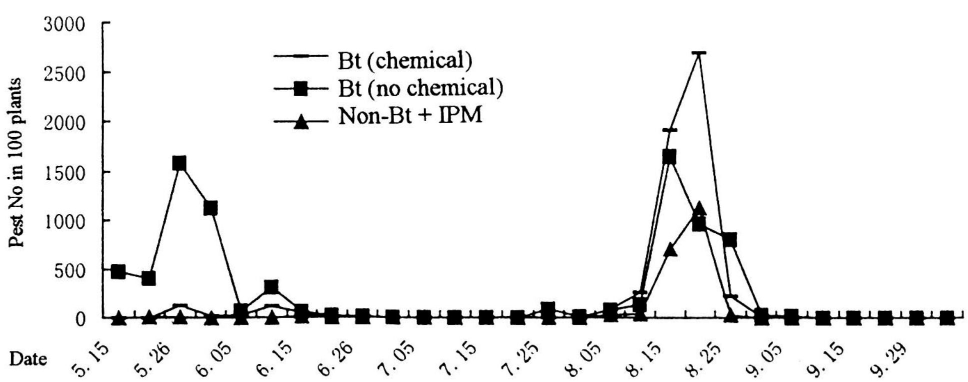
Graph 10 Population dynamics of Cotton aphids (Hubei, 2000)



B. Carmine spider mite (*Tetranychus cinnabarinus*)

In this study, the number of Carmine spider mites in *Bt* cotton was much higher than in non-*Bt* control cotton. This insect can largely threaten cotton's growth especially in its seedling stage. A field survey on May 26 revealed that the occurrence rate of Carmine spider mite found in *Bt* cotton was 85% and the season mean number of mites per 100 plants was 1580 individuals, while population levels in conventional cotton during the first peak were minimal (Graph 11). Another peak occurred from mid-August to early September with mite numbers of 2750 individuals in *Bt* plus chemical control, 1700 individuals in *Bt* without chemical control, and approximately 1000 individuals in the conventional non-*Bt* control fields (Zhang *et al.*, research report 2000).

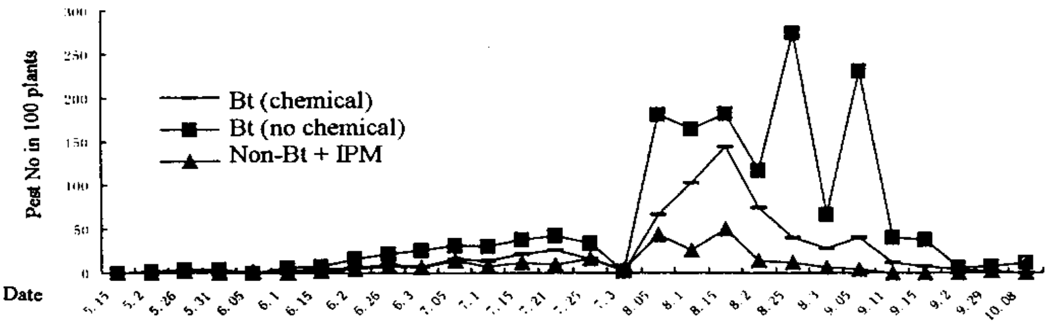
Graph 11 Population dynamics of Carmine spider mite
(Hubei, 1999-2000)



C. Cotton whitefly (*Trialeurodes vaporariorum)*

The number of cotton whiteflies in two *Bt* cotton fields was higher than that in non-*Bt* conventional cotton. Comparing the two *Bt*-cotton treatments, *Bt*-cotton without chemical controls had more whiteflies than *Bt*-cotton treated with chemicals (Graph 12).

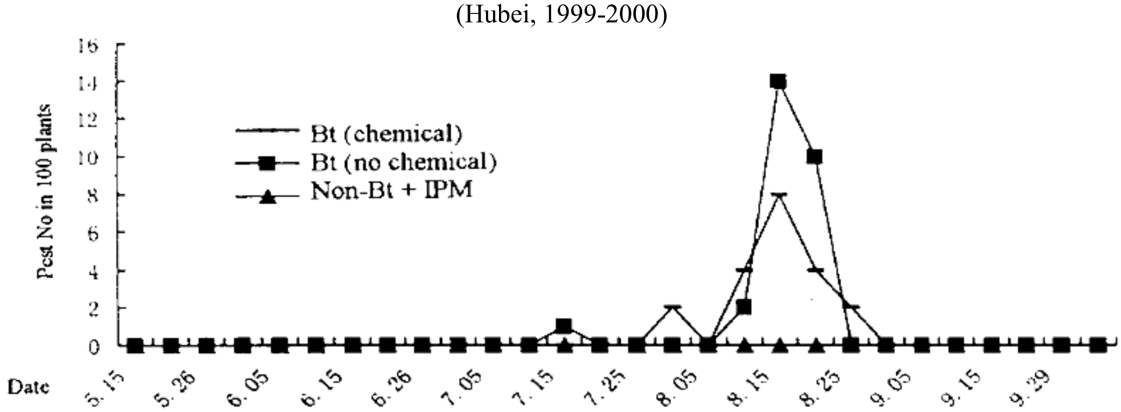
Graph 12 Population dynamics of cotton whitefly (*Trialeurodes vaporariorum)*
(Hubei, 1999-2000)



D. Cotton leafworm (*Prodenia litura* Fabticius)

There was little occurrence of this pest in non-*Bt* conventional cotton (+ IPM), but it was found in abundance in *Bt* cotton in the latter part of the season, with a peak in mid-August (Graph 13). The field survey found that the average daily leafworm number during the August peak was 14 individuals per 100 plants in *Bt* cotton (without supplementary chemical control), more than the number of cotton bollworms per plant found in the same fields at same period.

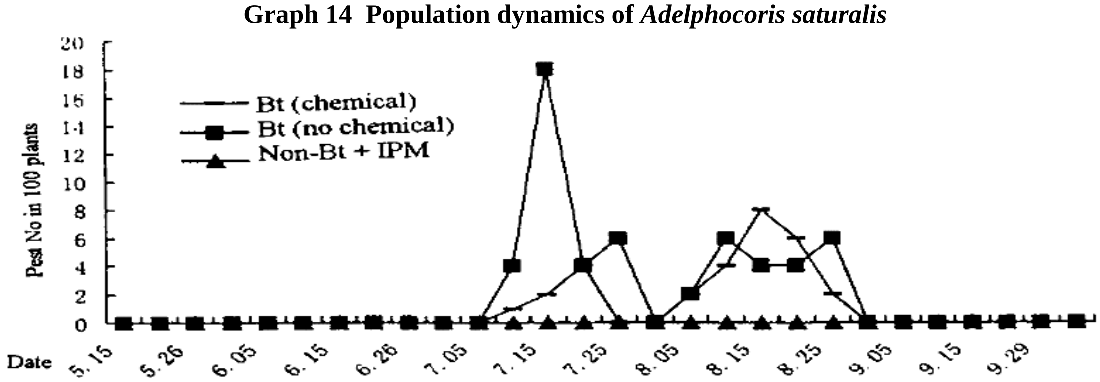
Graph 13 Population dynamics of cotton leafworm
(Hubei, 1999-2000)



E. Black-striped plant bug (*Adelphocoris saturalis* Jakovlev)

Similar to the cotton leafworm, there was little occurrence of the black-striped plant bug in conventional cotton (+ IPM). However, there were two population outbreaks in both the *Bt* treatments during July and August, where populations reached 18 individuals per 100 plants in the *Bt*-cotton not treated with chemicals (Graph 14).

Graph 14 Population dynamics of *Adelphocoris saturalis*



F. Onion thrips (*Thrips tabaci* Lindem)

As mentioned above, almost all important secondary pests had higher populations in *Bt* cotton than in non-*Bt* cotton for the field tests in Hubei Province during 1999 to 2000. However for the field tests in Hunei, there was no difference in population sizes of the two pests *Thrips tabaci* Lindem and *Frankiniella intomsa* Trybom in *Bt* cotton and non-*Bt* cotton fields. All three treatments had serious population outbreaks with the peak occurring from early July through the end of August. The number of individuals from the two species reached to 1800 to 4000 individuals per 100 plants; thrips population levels were higher in the non-*Bt* treatment than in the two *Bt* cotton treatments.

In another field test conducted by Prof. Zhang in Xinjiang there was a large peak of the thrips population in *Bt*-cotton grown without chemicals, with 2393 individuals in 100 plants. There was no thrips outbreak in the non-*Bt* conventional cotton field (Zhang *et al.*, research report 2000).

(4) Results from Prof. Shen Jinliang, Dept. of Plant Protection, Nanjing Agricultural University, Nanjing, Jiangsu

Field tests conducted in Cangshu and Funing of Jiang Province in 2001 demonstrated that *Bt* cotton (Xin 33B-Monsanto) not only was ineffective in controlling non-target sucking pests of cotton aphids, cotton spider mite, and lygus bugs, but also the population number and damage from these kinds of pests were greater that in non-*Bt* conventional cotton. For instance, in the Cangshu field test, the average damage rate of the second generation of lygus bugs was 60% per 100 plants in *Bt* cotton (33B-Monsanto) and chemical control was necessary, while the damage rate in the non-*Bt* control (Su Series) was only 35% (Shen, research report 2001).

3. Impacts on insect community diversity

Community diversity is measured in a number of ways. One measure of diversity is evenness – the distribution of individuals across species in a community. If all the species occurring in an area have similar population levels, the evenness of that community is quite high. Conversely, if one or a few species dominate with large populations, and most of the other species in the community have fairly low population levels, that community has low evenness. A measure of "unevenness" is dominance. Ecologists consider that communities with greater evenness will be more stable and pest species will therefore be less dominant. Fields with lower evenness may be less stable communities with increased potential for pest outbreaks or potential for some pests to become dominant. A composite measure of diversity often used is the Shannon diversity index, which takes into consideration both evenness and species richness (total number of species found).

Shannon diversity index

$$H' = -\sum_{i=1}^{s} P_i \ln P_i$$

Shannon evenness measure

$E = H'/\ln S$

Simpson's index (dominance)

$$D = \sum_{i=1}^{s} p_i^2$$

Where

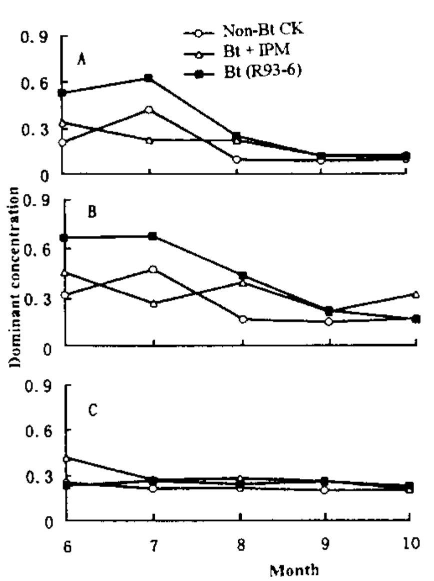
- $P_i = n_i/N$, the proportional abundance of the ith species

- n_i is the number of individuals in species i
- N is the total number of individuals
- S is the total number of species

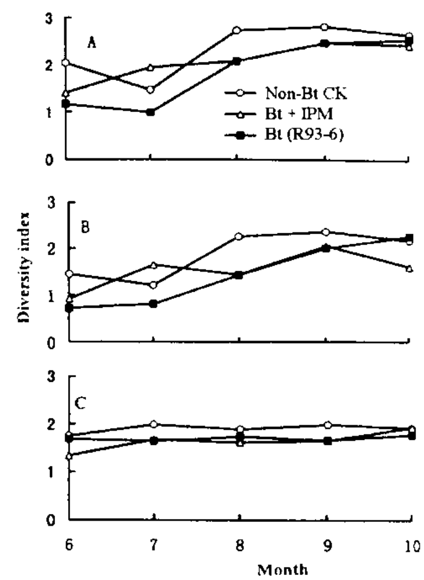
(1) Results from Cui Jinjie and Xia Jingyuan, Cotton Research Institute, CAAS. Anyang, Hebei.

The studies conducted during 1996-1997 were focused on the impacts of *Bt* cotton on insect community diversity, including a measure of species evenness within the community. The components of community diversity in transgenic *Bt* cotton were studied in three plots: *Bt* cotton (R93-6) plus integrated pest management (IPM); *Bt* cotton (with additional chemicals); and non-*Bt* cotton control (without chemicals). The study showed that Shannon diversity indices of insect, pest, and pest-natural enemy communities are lower in *Bt* cotton fields than in conventional cotton fields, while dominance is higher in *Bt* cotton than in conventional cotton. Given these figures, one could conclude that insect, pest and natural enemy sub-communities will be less stable in *Bt* cotton fields than in traditional non-*Bt* cotton fields (Graph 15), suggesting potential outbreaks of some pest species (Cui and Xia 2000a, 2000b).

Graph 15 Changes in dominance and Shannon diversity of insect community, pests and their natural enemy sub-communities



Fig(1) Changes in dominant concentrations of insect community (A), pest (B) and their natural enemy sub-communities (C) in the three cotton plots



Fig(2) Changes in diversitiy indexes of insect community (A), pest (B) and their natural enemy sub-communities (C) in the three cotton plots

(2) Results from Prof. Zhang Qingwen, Dept of Plant Protection, China Agricultural University, Beijing

Species diversity within a community is also used to predict community stability. Results from a study conducted in Xinjiang showed that species diversity was poorer in *Bt* cotton (MD-80) than in non-*Bt* cotton, with the species number of 56 in *Bt* cotton field and 67 in the non-*Bt* control (Table 6). These results suggest that *Bt* cotton may have a negative impact on species diversity. (Zhang *et al.*, research report 2000).

Table 6 Insect species diversity in different cotton fields (Xinjiang, 1999)

Treatments	Pest species (No.)	Natural enemies (No.)	Spiders (No.)	Total (No.)
Bt cotton (without IPM)	28	20	8	56
Non-*Bt* control (without IPM)	30	18	19	67

Similar to the results from Dr Cui and Prof. Xia in Anyang, the results of this study also showed a higher insect dominance in *Bt* cotton than in the non-*Bt* control (Table 7).

Table 7 Insect dominance in different cotton fields (Xinjiang, 1999)

Treatments	Insect community	Natural enemies Sub-community	Pest sub-community
Bt cotton	0.9264	0.2082	0.9453
Non-*Bt* control	0.6881	0.1593	0.7537

These results imply that insect community stability in *Bt* cotton fields may be much reduced compared to non-*Bt* cotton fields. A higher insect dominance in *Bt* cotton than in the non-*Bt* control suggests an increased potential for pest outbreaks for certain pest species in *Bt* cotton fields.

Another field test was conducted in Julu, Hebei Province in 1998 by Wei Guoshu and Zhang Qingwen. The study was sponsored by Monsanto Company. The treatments were *Bt* cotton (33B-Monsanto), *Bt* cotton (Zhong 30), non-*Bt* cotton with supplementary chemical control and non-*Bt* cotton with no additional chemicals used. The study focused on comparing the impacts of the treatments on arthropod community structure. The results showed that the species diversity in both *Bt* treatments was lower by 2.4% to 16.3% and population numbers reduced by 71.0% to 78.3%, compared with the non-*Bt* cottons (Wei *et al.*, 2001) (Table 8).

Table 8 Impacts of *Bt* cotton on species diversity of arthropods (Hebei 1998)

Species diversity indices	*Bt* cotton (33B-Monsanto)	*Bt* cotton (Zhong 30)	Non-*Bt* cotton (with chemical)	Non-*Bt* cotton (without chemical)
Number of arthropod orders	14	14	14	15
Number of arthropod families	26	29	31	36
Number of arthropod species	41	41	42	49
Number of arthropod individuals	18939	14218	65407	15287

RESISTANCE OF COTTON BOLLWORM TO *BT* COTTON

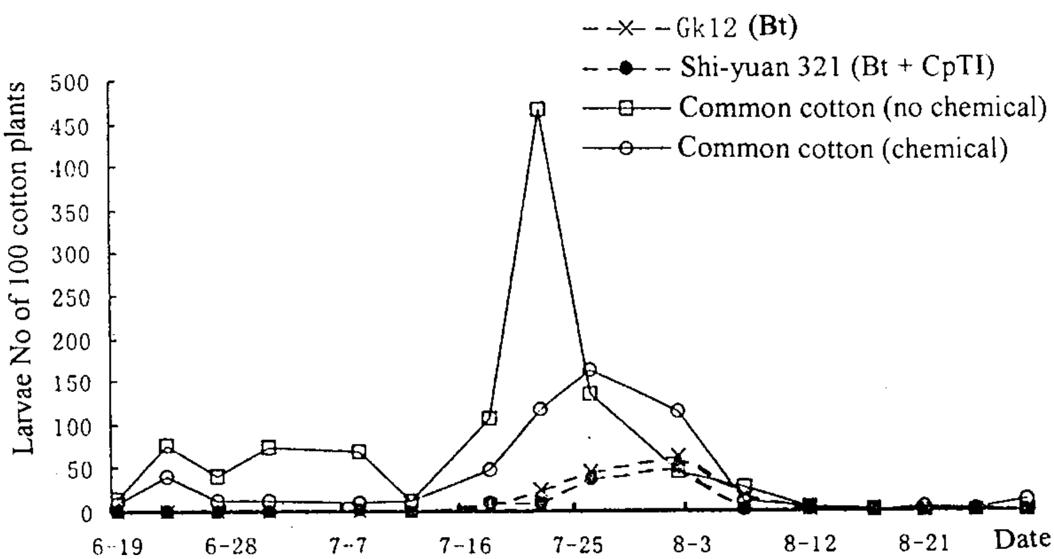
1. Resistance Development of Cotton Bollworm

It is commonly recognized that *Bt* cotton effectively controls the second generation of cotton bollworm (*Helicoverpa armigera*) (the first generation uses other crops, such as wheat, as hosts), but it is less effective in controlling the third and fourth generations. Chemicals or non-chemical IPM methods are necessary in *Bt* cotton fields after mid-July or early August to control the third and the fourth generations, regardless of whether the fields are found in North China or South China. Total chemical applications in cotton have been reduced from 13 sprays per season to 7 sprays in the Yellow River Valley area and from 5-7 sprays down to 3-5 in the Yangtse River Valley area. As labor is saved through reduction of chemical applications, farmers currently favor *Bt* cotton.

(1) Results from researchers in Institute of Plant Protection, CAAS, Beijing

Wu (2000) reported from his field study in Langfang, Hebei in 2000 that in all *Bt* cotton treatments, whether containing a single *Bt* gene (GK 12) or two genes (Shiyuan 321, *Bt* + CpTI), there was a small peak of cotton bollworm occurrence between mid-July and early August (Graph 16). The peak population of bollworm in *Bt* cotton reached 50 individuals in 100 plants, and chemical control was necessary (Wu 2000)

Graph 16 Population dynamics of cotton bollworms in different cotton fields
(Langfang, Hebei, 2000)



Liang *et al.* (2000a) used *Bt*-resistant bollworm produced in the laboratory to screen for and study the inheritance of resistance to *Bt* transgenic cotton in cotton bollworm. The lab test results showed that after 16 generations of selection, the WLC_{50} (mean weight loss concentration) of cotton bollworm feeding on *Bt* transgenic cotton was 43.3 times less than in the first generation. After studying the degree of dominance by cross and reciprocal-cross between the resistant and susceptible populations, the authors suggest that the inheritance of resistance to *Bt* transgenic cotton in cotton bollworm is controlled by a single autosomal incompletely recessive allele (Liang *et al.*, 2000a).

In other work, Liang *et al.* (2000b) used laboratory-screened bollworms which are resistant to *Bt* pesticide, *Bt* protoxin and *Bt* transgenic cotton to study the cross-resistance in these *Bt* resistant populations respective to other *Bt* preparations, chemical insecticide and fresh *Bt* transgenic cotton. The results were as follows: After selection for 16 generations, the cotton bollworms were more resistant to *Bt* pesticide than *Bt* protoxin and *Bt* transgenic cotton, with resistance ranked as follows: *Bt* pesticide > *Bt* protoxin > *Bt* transgenic cotton. The authors found in cross-resistance tests that when cotton bollworms developed resistance to a certain *Bt* preparation, they had a positive cross-resistance to other preparations containing the same *Bt* gene, but only negligible or negative cross-resistance to preparations containing a different *Bt* gene. The same results were obtained in tests with fresh cotton leaves from field. In the resistant populations, the seven-day old larvae were sensitive to deltamethrin, fenvalerate, phoxim and endosulfan, while reactions to these chemicals of the 3^{rd} instar larvae in different populations were irregular. When fed with fresh leaves of *Bt* transgenic cotton, the mortality of the larvae from the population already resistant to *Bt* transgenic cotton decreased significantly (Liang *et al.*, 2000b).

In laboratory experiments, Zhao (1998) showed when screening reached the 11^{th} and 17^{th} generation, the index of resistance of bollworm larvae to *Bt* protoxin would increase by 4 times and 7.1 times respectively. Zhao anticipated that control of bollworm by *Bt* transgenic cotton would decrease noticeably after *Bt* cotton had been planted on a large scale for 6 years, and that control would be lost after *Bt* cotton had been planted on a large scale for 9 years. These findings are in accordance with results from computer modeling done in Australia, which predicted that *Bt* transgenic cotton could be effectively used for only 8-10 years (Zhao 1998).

Kongming *et al.* (2002) conducted field research during 1998-2000, measuring sensitivities of field populations of cotton bollworm, *Helicoverpa armigera* (Hübner), to *Bt* insecticidal protein Cry1Ac. Forty-one populations were sampled, most of them collected from the *Bt* cotton planting region. The researchers measured the concentration of Bt that produced 50% inhibition of larval development to 3rd instar (IC_{50}) among different populations; the range of IC_{50} values measured over three continuous three years were 0.020 μg/ml - 0.105 μg/ml, 0.016 μg/ml - 0.099μg/ml and 0.016 μg/ml - 0.080 μg/ml, respectively. The results from discriminating dose studies (IC_{99}) showed the proportions of individuals reaching 3rd instar ranged from 0% to 4.35%, suggesting that the field populations sampled were still susceptible to Cry1Ac protein.

(2) Results from researchers in Cotton Research Institute, CAAS, Anyang, Hebei.

1997-2000, Cui X. *et al.* (2001a) collected cotton bollworm from experimental fields. After being fed an artificial diet mixed with *Bt* toxin protein (Cry1Ac), the bollworms were screened for 16 to 21 generations in the laboratory. The researchers measured the amount of Bt toxin needed to kill 50% of the individuals (LC_{50}) and found the value of the LC_{50} in the 19^{th} generation (4.3646 g L^{-1}) increased 14.7-fold over the LC_{50} in the first generation.

Further research was conducted by Cui X. *et al.* in 2001 (2001b). This study focused on laboratory comparison of the reactions to *Bt* toxin proteins of cotton bollworm strains from Anyang, Henan and Xinjiang. The results showed that the LC_{50} of the bollworm strain in Anyang was 8.7645g L^{-1}, 34 times that of the Xinjiang bollworm strain whose LC_{50} was only 0.2547g L^{-1}. Larvae of the Xinjiang bollworm strain at most developed to the fourth instar in *Bt* transgenic cotton with 6.7% of the individuals surviving to the fourth instar. However, larvae of the Anyang bollworm strain finished their entire growth on *Bt* cotton, with a 45.5% survival rate for 4^{th} instar larvae, 12.6% for 5^{th} instar and 3.8% for 6^{th} instar respectively. 1.6% of Anyang bollworm larvae developed to the nymph phase (Cui X. *et al.*, research report 2001b). The authors suggest that the cotton bollworm in Anyang, Henan has already developed resistance to *Bt* cotton as *Bt* cotton has been planted continuously in Anyang for several years. Xinjiang has no history of *Bt* cotton use; *Bt* cotton was just introduced recently with only 1% of the cotton area being planted to *Bt* cotton in 2000. Xinjiang accounted for about 25% of all cotton planted in China.

(3) Results from Prof. Shen Jinliang, Dept of Plant Protection, Nanjing Agricultural University, Nanjing, Jiangsu.

Shen *et al.* have been studying resistance of cotton bollworm to *Bt* since 1995 (Shen *et al.*, 1998). Their laboratory work during the past four years has shown that under the condition of fresh *Bt* cotton feeding, the cotton bollworm will develop a measurable resistance to *Bt* cotton. Shen's experiments demonstrated that *Bt* cotton control of bollworm would be decreased from 100% to 30% when the 17th generation of the selected cotton bollworm was fed *Bt* cotton at the seedling stage. At the 40th generation, the resistance of the bollworm strain is increased 500-1000 times compared to the original population.

In field tests conducted in Funing, Jiangsu Province in 2001, Shen *et al.* demonstrated that Xin 33B (*Bt*-Monsanto) provided effective control of the second generation of cotton bollworms in the fields, while control efficiency of the third and the fourth generations was decreased. Relative to control of second generation bollworm, the number of larvae found per 100 plants and the bud damage rate increased for the 3rd and 4th generations of cotton bollworm (Table 9) (Shen, research report 2001).

Table 9 Result of the field test for cotton bollworm control (Funing, Jiangsu 2001)

Treatments		The 2nd bollworm		The 3rd bollworm		The 4th bollworm	
		CBW No /100 Plant	Bud No. damaged	CBW No /100 Plant	Bud No. damaged	CBW No /100 Plant	Bud No. damaged
Bt cotton (33B)		0	0	2	4	4	6
CK	CK (Su 9)	8	18	5	12	10	29
	CK (Su 12)	8	20	6	15	7	23
	CK (Su 15)	4	9	7	21	11	30
	CK (average)	6.67	15.67	6	16	9.3	27.3

2. Discussion of bollworm resistance to transgenic *Bt* cotton

Some experts propose that bollworm resistance to *Bt* cotton could be avoided if a high-dose expression of the *Bt* toxin protein can be maintained in *Bt* cotton. Others consider that resistance of bollworm may be postponed if a "refuge" mechanism is also used, by planting other crops such as corn in or around *Bt* cotton fields in order to maintain a population of homozygous susceptible moths for mating with any resistant moths that may develop. However, some scientists have challenged the science behind these resistance management strategies, and many laboratory tests and field studies have supported these challenges. (for useful reviews of these proposals and the scientific challenges to the proposals see Andow 2002 and Gould 1998)

(1) Transgenic Bt has limited crystal insecticidal protein expression. Maintaining a high-dose is difficult.

Using ELISA and laboratory bioassays, Zhang Yongjun *et al.* (2001), from the Plant Protection Institute of CSSA, studied the relationship between the *Bt* insecticidal protein expression levels in *Bt* transgenic cotton and the mortality of cotton bollworm. The results showed that there were obvious spatio-temporal changes in the content of *Bt* insecticidal protein coinciding with developmental processes in *Bt* cotton. Generally, *Bt* protein concentrations increased during development from seedling to flowering and boll-formation, but after that concentrations decreased through boll opening. Higher concentrations were found in the leaf and flower heart and square, with lower concentrations in the boll, petals and bracts. Larval mortalities in indoor bioassays were correlated with *Bt* toxin concentrations throughout time and throughout the plant. The researchers suggest that owing to the decrease of *Bt* insecticidal protein content in the middle to late growing period of *Bt* cotton, population monitoring should be strengthened and complementary chemical control used against 3rd and 4th generation cotton bollworm (Zhang *et al.*, 2001).

Shen (pers. comm. 2001) notes that *Bt* cotton will never have a high dose of the toxin in cotton's late growth stage. It only expresses a high dose in young cotton. This may explain why some bollworms survive in *Bt* cotton and cause damage in the third and fourth generations.

Shen (pers. comm. 2001) also concluded that bollworms developed resistance more quickly on transgenic *Bt* cotton than with topical *Bt* sprays, perhaps because *Bt* spray contains several insecticidal crystal proteins and insect exposure to the toxin is only for a short time. Transgenic *Bt* cotton contains only one insecticidal crystal protein (Cry1Ac) and the toxin is expressed throughout the entire growing season.

(2) Refuge mechanism is doubtful

A system of refuges has been proposed to prolong resistance development by facilitating mating between resistant individuals and susceptible ones found in refuges or in nearby fields of other crops such as corn. If the resistant gene is an autosomal incompletely recessive allele, then theoretically by planting a certain area of non-*Bt* cotton or other crops, resistant cotton bollworms (R) developing in *Bt* cotton could mate with *Bt* susceptible individuals (S) to maintain production of susceptible heterozygotes (RS), and prevent RR matings.

However, it will be difficult to implement a refuge system under the situation of small-plot cultivation in China. In most provinces, the farmers possess a very small piece farmland, usually only 0.1 to 1.0 ha for one family. Incorporation of a refuge in the cropping system is not realistic. Actually, when 1 or 2 families begin to plant *Bt* cotton in a village, other families usually follow. In this situation it would be difficult to maintain an adequate number of small plots as refuges.

Additionally, the mechanism itself may be theoretically flawed. Shen (pers. comm. 2001) has commented that the mating period for adult bollworm may not be synchronous between the bollworms in *Bt* cotton and common cotton. The larval period in *Bt* cotton can be prolonged to 21 days while it is only approximately 15 days in conventional cotton. A delay in the larval stage of 5-6 days would mean that adults developing on *Bt* cotton could only mate with each other, rather than the adults developing on the refuge cotton or in other crops. Mating normally takes place over a 2-3 day period (Shen, personal communication; see also Liu *et al.* 1999 for a discussion of the same phenomenon in pink bollworm).

Studies done by Cui and Xia also support this hypothesis. Their laboratory studies document the behavior of cotton bollworm fed *Bt* cotton. The results showed the time of spin-down and staying was prolonged considerably and feeding time substantially decreased, leading to a delay in growth and development of bollworm larvae (Cui and Xia 1998).

(3) Geographical suitability of Bt cotton

As *Bt* cotton provides good control of second generation cotton bollworm, it is more effective in North China, because the first and second generations of cotton bollworm are serious cotton pests in the North China region. On the other hand, *Bt* cotton is less effective in the Yangtse River Valley because the third and fourth generations are the more serious cotton pests in this region, and *Bt* cotton is not so effective on the third and fourth generations. *Bt* cotton has geographical limitations to its use.

Based on research monitoring the resistance of cotton bollworm to *Bt* cotton during 1995 to 1997, Zhao (1998) found that the susceptibility of cotton bollworm to *Bt* crystal insecticidal protein varied among geographical regions. Bollworm is most susceptible to *Bt* cotton in the Xinjiang Autonomous Region and there is a lower frequency of resistant individuals in the North China region (Zhao 1998).

Wu *et al.* (1999) studied geographic variation China in susceptibility of cotton bollworm to *Bt* insecticidal protein. Bollworm were collected from 5 different ecological regions where cotton is grown, and dose responses to Cry1Ac protein of mortality and growth inhibition were evaluated. The ranges of LC_{50} and IC_{50} of 3rd instar larvae from the five different geographical areas were 0.091-9.073 ∝g/ml and 0.011-0.057 ∝g/ml respectively (Wu *et al.* 1999) A more recent study of 41 populations found LC_{50} concentrations ranging from 0.016-0.099 µg/ml. (Wu *et al.* 2002)

In addition, there is an important differences between the target pest species in the Yellow River Valley area and the Yangtse River Valley area. Cotton bollworm is the only very important target pest for cotton production in Yellow River Valley area (North China), whereas there are two target pest species in Yangtse River Valley area (South China): cotton bollworm and cotton pink bollworm (*Pectinophora gossypiella* Saunders). Therefore, it is important to consider both geographical suitability and pest differences when *Bt* cotton is popularized.

(4) Transgenic cottons with two insecticidal genes

To prevent development of bollworm resistance to *Bt* cotton, scientists are developing transgenic *Bt* cotton with two crystal insecticidal protein genes, or two genes coding for unrelated insecticidal proteins. Experiments conducted by Wu (2000) in Langfang, Hebei showed the double-gene (*Bt* + CpTI - Cowpea trypsin inhibitor) cotton (Shiyuan 321) showed no indication of a high-dose *Bt* expression (Wu, research report, 2001). There was a peak for bollworm occurrence mid- July to mid-August for double-gene cotton (Shiyuan 321, *Bt* + CpTI), the same as for single-gene *Bt* cotton (GK12) (see Fig.16).

Studies in Anyang also verified that double-gene cotton gave no advantage over single-gene cotton (Cui *et al.*, unpublished report, 2001). Results indicated that double-gene cotton (*Bt* + CpTI) provides weak control over blank cutworm (*Agrotis ypsilon* Rottemberg) in early instar stages compared to single-gene cotton (*Bt*), but it provides greater control over later instars of the pest than *Bt*-alone cotton.

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