



# Impacts of transgenic poplar-cotton agro-ecosystems upon target pests and non-target insects under field conditions

D.J. Zhang<sup>1,2\*</sup>, J.X. Liu<sup>1\*</sup>, Z.Y. Lu<sup>3</sup>, C.L. Li<sup>1</sup>, E. Comada<sup>4</sup> and M.S. Yang<sup>1</sup>

<sup>1</sup>College of Forestry, Agricultural University of Hebei, Hebei, China

<sup>2</sup>College of Life Science, Inner Mongolia University, Huhhot, China

<sup>3</sup>Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Huhhot, China

<sup>4</sup>Chinese Culture Center of San Francisco, San Francisco, CA, USA

\*These authors contributed equally to this study.

Corresponding author: M.S. Yang

E-mail: yangms100@126.com

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**ABSTRACT.** Poplar-cotton agro-ecosystems are the main agricultural planting modes of cotton fields in China. With increasing acres devoted to transgenic insect-resistant poplar and transgenic insect-resistant cotton, studies examining the effects of transgenic plants on target and non-target insects become increasingly important. We systematically surveyed populations of both target pests and non-target insects for 4 different combinations of poplar-cotton eco-systems over 3 years. Transgenic Bt cotton strongly resisted the target insects Fall webworm moth [*Hyphantria cunea* (Drury)], *Sylepta derogata* Fabricius, and American bollworm (*Heliothis armigera*), but no clear impact on non-target insect cotton aphids (*Aphis gossypii*). Importantly, intercrops containing transgenic Pb29 poplar significantly increased the inhibitory effects of Bt cotton on Fall webworm moth in ecosystem IV. Highly resistant Pb29 poplar reduced populations of the target pests *Grnsonoma*

*minutara* Hubner and non-target insect poplar leaf aphid (*Chaitophorus populiabae*), while Fall webworm moth populations were unaffected. We determined the effects of Bt toxin from transgenic poplar and cotton on target and non-target pests in different ecosystems of cotton-poplar intercrops and identified the synergistic effects of such combinations toward both target and non-target insects.

**Key words:** Bt toxin; Insect-resistant gene; Non-target insects; Target pests; Transgenic poplar-cotton eco-system

## INTRODUCTION

Crops produced by genetic modification conferring pest or disease resistance play an important role in crop production (Perlak et al., 1990). Insect-resistant transgenes largely rely on the transformation of insecticidal toxin genes from microbes to target organisms, with the *Bacillus thuringiensis* (Bt) toxin gene most widely used. The Bt endotoxin is encoded by various *Cry* genes and have specific activities against insect species of different orders, typically by paralyzing the digestive tract of pests (Gill et al., 1992). Because of its selective activity toward their target insects, transgenic crops allow for reduced usage of broad-spectrum insecticides, thus contributing to the conservation of insect communities from an ecological perspective (Simeon et al., 2005). Major insect-resistant crop varieties such as cotton, rice, maize, soybean, and wheat have been developed, some of which have passed field and environmental release trials and now were broadly cultivated on a commercial scale (Christou et al., 2006).

With the development of transgenic organisms and their large-scale commercial application, the bioefficacy of these organisms against target pests has been extensively examined and the biosafety of transgenic plants on human health and the environment has received worldwide attentions (Verna et al., 2011). The relative efficiency of Bt toxin toward target pests has been evaluated in laboratory, semi-field, and field studies; the data suggest that this efficiency varied largely because of temporal and spatial fluctuations of toxin gene expression during different growth stages, various plant parts, and varying environmental conditions such as relative humidity, soil salinity, and waterlogging (Adamczyk Jr. and Gore, 2004; Liu et al., 2005; Jiang et al., 2006; Luo et al., 2008; Chen et al., 2012). Additionally, the susceptibility of insects to the toxin was influenced by their feeding behavior and also diverged in different life stages (Wu et al., 2002; Men et al., 2005). Thus, Bt toxin level variation in transgenic plants and the relative vulnerability of insects constitute a complex interaction between the crop and the pests. However, the Bt toxin expressed by transgenic plants can also produce specific effects on individuals and populations of non-target insects as well as on the entire insect community, and may affect functions of the entire ecological system; deleterious effects on target pests have also been reported (Chen et al., 2007). Various field studies have been conducted to investigate the changes in abundance and diversity of target and non-target taxa in the presence of different transgenic crops. Some studies have also suggested a number of mechanisms by which Bt plants may affect an insect community from the perspective of food web levels, including predator/prey and host/parasitoid systems (von Burg et al., 2011; Xu et al., 2012). However, most of these studies used single farmland or forestland as the subject, while comparatively less attention has been focused on defining the effects of intercrops and

agro-ecosystem on insect populations. Therefore, it is important to analyze potential impacts of transgenic plants on the insect community and assess the ecological risk resulting from these impacts.

Ecosystems are very complex, and the understanding of the numerous interactions between organisms in agro-ecosystems is limited. Therefore, the research gap in this field must also be addressed to determine interactions between agro-ecosystems and insect biodiversity. Previous studies have examined the effects of different combinations of intercrops on insect community composition (Cai et al., 2010; Yao et al., 2012). The poplar-cotton ecosystem is the main agricultural planting mode in cotton regions of China. Transgenic cotton producing Bt toxin Cry1Ac has been planted in the USA since 1996 and has been commercially available in China since 1997 (Clive et al., 2006). Transgenic poplar carrying the Bt toxin gene was also developed and commercialized (Rao et al., 2001). With the introduction of transgenic poplar and transgenic cotton into fields, these transgenic plants co-existed and interacted in a single ecosystem. Because the performance and interactions of transgenic crops are complicated, the bio-efficacy and biosafety of this system need to be addressed. Moreover, the combination of forestland and farmland has attracted less attention in agroforestry ecosystems. Therefore, we evaluated the relative efficacy of a transgenic agroforestry system on their target pests as well as the non-selective impact of such an ecosystem on non-target insects. We investigated both the total abundance and seasonal dynamics of target and non-target insect species on cotton fields and poplar trees in different agro-ecosystem backgrounds. Lepidopteran caterpillars are the target pests of Bt toxins (Stewart et al., 2001). The lepidopteran target insect Fall webworm moth, *Sylepta derogata* Fabricius, *Heliothis armigera*, *Grnsonoma minutara* Hubner, and non-target hemipteran insects *Aphis gossypii* and *Chaitophorus po-pulialbae* were investigated. Our results for the transgenic poplar-cotton agro-ecosystem will also help in the monitoring of the long-term impact of transgenic ecological systems on insect resistance development.

## MATERIAL AND METHODS

### Materials

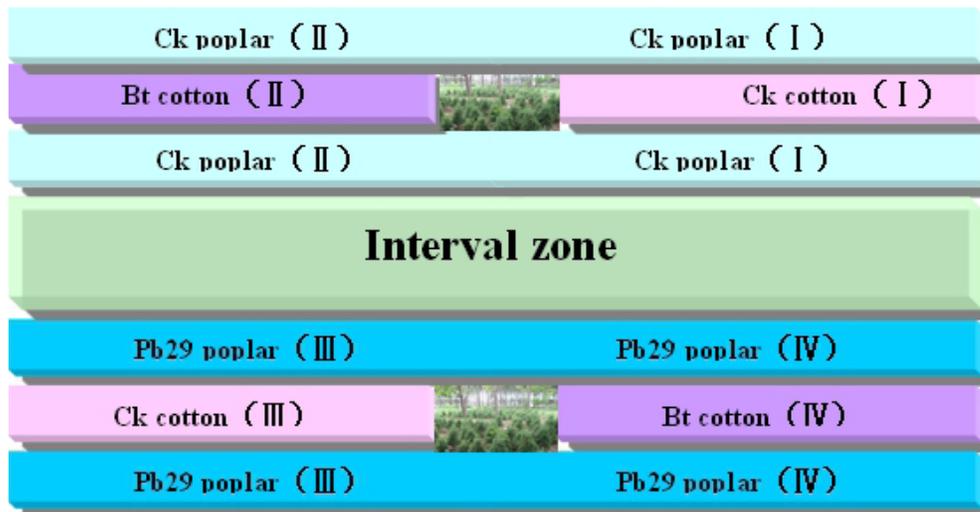
The plants were provided by College of Forestry, Agricultural University of Hebei. The plants included non-transgenic poplar 741 (Ck poplar), a highly insect-resistant transgenic strain Pb29 of 741 poplar (high-resistant poplar Pb29), non-transgenic cotton - Xinluzao No. 36 (Ck cotton), and transgenic cotton - Handan 109 (Bt cotton). Poplar trees were planted in March 2005 in this study.

Poplar 741 was cultivated as a superior white poplar hybrid clone by the Agricultural University of Hebei. The hybrid combination was [*Populus alba* L. x (*P. davidiana* Dode. + *P. simonyi* Carr.)] x *P. tomentosa* Carr (Jiang et al., 1991). Transgenic hybrid poplar 741 was obtained by transformation of the Bt insecticidal protein gene (BtCry1Ac) and the arrowhead proteinase inhibitor gene into *Populus tomentosa* 741 clones through the *Agrobacterium*-mediated method (Yang et al., 2006). Pb29 line is one most highly insect-resistant strains and has been commercially licensed to for cultivation in controlled areas as approved by the National Forestry Bureau of Biological Gene Engineering Safety Commission in 2004. Xinluzao No. 36 (Xinshi K8) was obtained through sexual hybridization breeding by Xinjiang Shihezi Cotton Research Institute in 1997. The female parent was a hybrid of the self-fertile and high-

yield early-maturing strain 1304. The male parent was disease-resistant strain BD103 from the Plant Protection Institute of Chinese Academy of Agricultural Science. Handan 109 was industrially bred as a new high-yield variety of BtCry1A expressing disease-resistant cotton by the Handan Academy of Agricultural Science and the Biotechnology Research Institute of Chinese Academy of Agricultural Science (Ban et al., 2005).

### Site description

The experimental release field was approved by the State Forestry Bureau of the Biological Genetic Engineering Safety Committee. The experimental forest was approximately 1 hm<sup>2</sup> at the national forest cultivation area in Zhuozhou city, Hebei Province, China (39°21'-39° 36' N, 115° 44'-116° 15'E). This area has a warm, temperate, semi-humid monsoon with typical continental monsoon climate. The annual average temperature is near 11.6°C. The annual average ground temperature is 14.2°C and the annual average depth of frozen soil is 40 cm. The area receives an annual average of 2569 h sunshine, annual average rainfall of 617 mm, and frost-free season of approximately 178 days. Ck poplar and high-resistant poplar Pb29 samples were planted in a plot that was approximately 25 x 100 m and the interval zone between samples was 100 x 100 m. Next to each of the poplar fields, Ck cotton and Bt cotton were planted in a 25 x 50-m region with oriental arborvitae as the interval zone between different types of cotton fields. Thus, the test field was divided into 4 distinct agro-ecosystems: Ck poplar and Ck cotton, Ck poplar and Bt cotton, high-resistant Pb29 poplar and Ck cotton, and high-resistant Pb29 poplar and Bt cotton. The corresponding sample numbers assigned to the 4 systems were I, II, III, and IV (Figure 1). Normal agronomic practices were followed to raise the crop. No insecticides were applied during the cropping season.



**Figure 1.** Profile of mixed planting pattern. Poplars were planted in an approximately 25 x 100-m area; the interval zone between samples was 100 x 100 m. Neighboring each poplar field, cotton was planted in an approximately 25 x 50-m region with oriental arborvitae in the interval zones between different types of cotton fields. Hence, the test field was classified into 4 distinct agro-ecosystems: Ck poplar and Ck cotton (I); Ck poplar and Bt cotton (II); high-resistant Pb29 poplar and Ck cotton (III); and high-resistant Pb29 poplar and Bt cotton (IV).

## Experimental designs and sampling methods

The transgenic poplar-cotton ecosystem was investigated for 3 years from June to October in 2009, 2010, and 2011. Cotton was sown on April 25-May 1 and harvested from September 20-October 1. Sampling was conducted on an average of every 10 days and continued until the cotton was harvested. The 50-cm long branches were randomly selected from 5 poplar trees in the poplar forest. The number of target insects and non-target insects was counted on the trunk surface of the main stem within 5 m of the ground surface. Five samples (0.5 x 0.5 m plot) were randomly collected from each sample of Ck cotton and Bt cotton. The species present and the number of target and non-target pests were determined and recorded for all cotton strains from each plot. Aphids were collected and counted from the third branch leaves of the top of the specimen.

## RESULTS

### Effects of transgenic poplar-cotton ecosystem on target insect Fall webworm moth

Transgenic poplar-cotton ecosystems had significant inhibitory impacts on Fall webworm moth populations in 2009 and 2010 (Table 1). Over these sampling years, the Fall webworm moth population showed a repeating pattern in the cotton field of ecosystem I-IV, i.e., system I > system III > system II > system IV. This pattern indicates that transgenic Bt cotton had strong inhibitory effects on the target insect Fall webworm moth compared with in control cotton; importantly, the intercrops of cotton fields of transgenic Pb poplar contained a dramatically decreased number of Fall webworm moth in the cotton field of same ecological system compared with intercrops of control poplar. In 2009, the seasonal dynamics of Fall webworm moth populations showed that the populations gradually increased and reached a maximum in late October in the Ck cotton field of systems I and III, among which an increase in the population appeared earlier in system I and reached higher numbers compared with system III. In 2010, the outbreak of Fall webworm moth appeared earlier in the cotton fields of system I and III, indicating an earlier peak in insect numbers compared with systems II and IV (Figure 2).

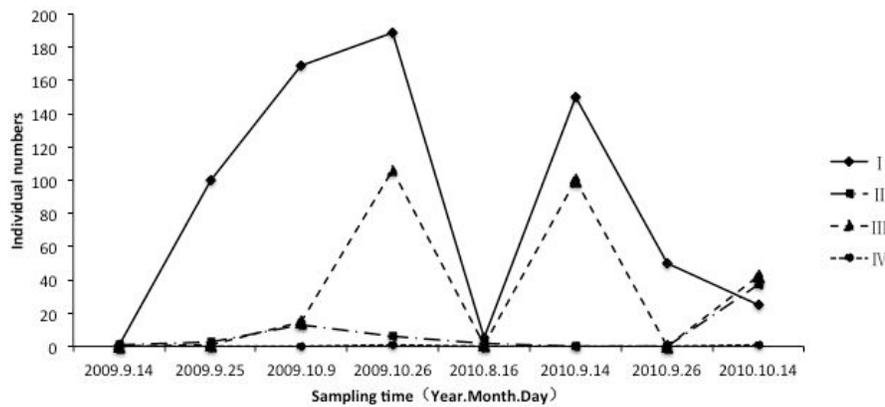
**Table 1.** Fall webworm moth population of transgenic poplar-cotton ecosystem (number).

| Year | Sample area | I   | II   | III  | IV  |
|------|-------------|-----|------|------|-----|
| 2009 | Cotton      | 459 | 23   | 122  | 1   |
|      | Poplar      | 59  | 583  | 1489 | 589 |
| 2010 | Cotton      | 230 | 39   | 143  | 1   |
|      | Poplar      | 622 | 1331 | 680  | 649 |
| 2011 | Cotton      | -   | -    | -    | -   |
|      | Poplar      | -   | -    | -    | -   |

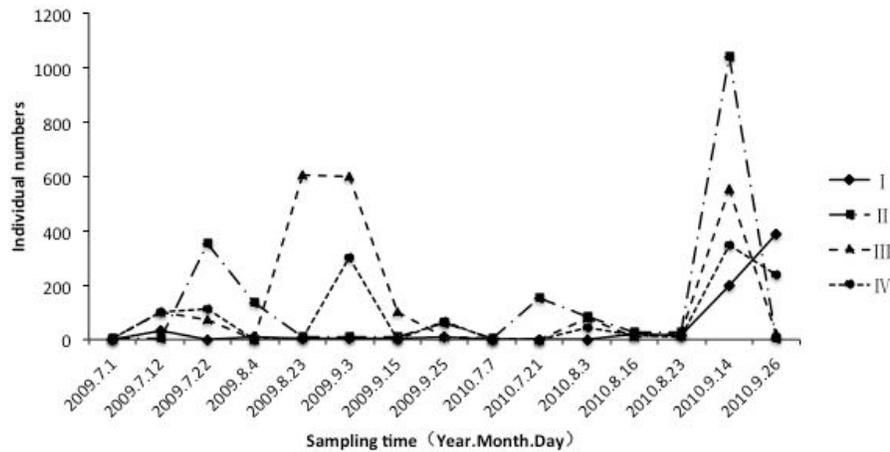
I: Ck poplar and Ck cotton; II: Ck poplar and Bt cotton; III: high-resistant Pb29 poplar and Ck cotton; IV: high-resistant Pb29 poplar and Bt cotton.

The abundance of Fall webworm moth was not correlated with the Bt transgene in the poplar field in 2009 (Table 1 and Figure 3), whereas the overall number on Pb29 poplar was comparable to or even higher than that on Ck poplar. In 2010, the numbers of Fall webworm moths in ecosystems I, III, and IV were similar, but the population was doubled in ecosystem II, demonstrating that the insects may have moved from the Bt cotton field to the poplar field.

Seasonal dynamic data showed that outbreak of Fall webworm moth in the cotton field consistently lagged behind the outbreak in the poplar field, supporting that Fall webworm moth in the cotton field moved from the poplar field in same ecosystem. These data illustrate that the transgene Bt toxin in cotton had a dominant inhibitory role in the intercrop system of transgene cotton and poplar ecosystem.



**Figure 2.** Seasonal dynamics of Fall webworm moth in the cotton field of transgenic poplar-cotton ecosystem. Ecosystems I-IV are described in Figure 1. Sampling of Fall webworm moth is described in Material and Methods.



**Figure 3.** Seasonal dynamics of Fall webworm moth in the poplar field of transgenic poplar-cotton ecosystem. Ecosystems I-IV are described in Figure 1. Sampling of Fall webworm moth is described in Material and Methods.

### Effect of transgenic poplar-cotton ecosystem on target insects *S. derogata* Fabrieius and *G. minutara* Hubner

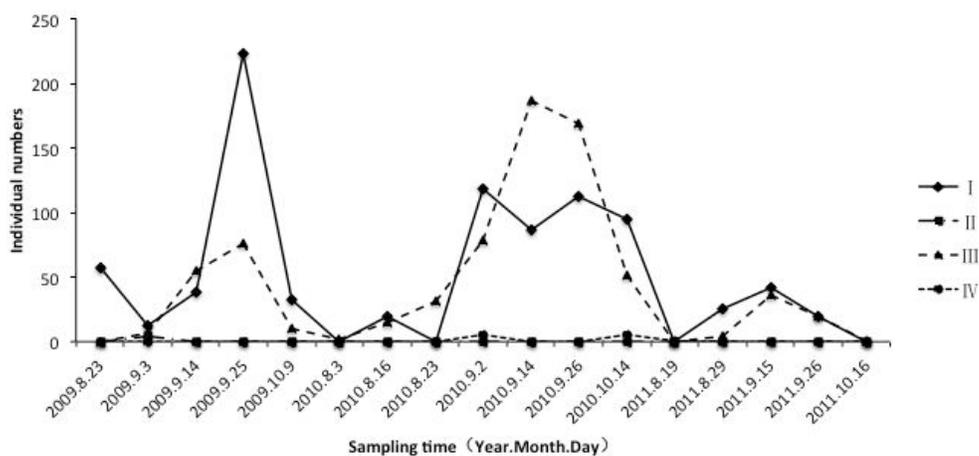
*S. derogata* Fabrieius and *G. minutara* Hubner belong to the lepidopteran family, which is the target of Bt toxin. *S. derogata* Fabrieius inhabits cotton and *G. minutara* Hubner is a pest of poplar.

The population of *S. derogata* Fabricius was consistently decreased in the transgenic cotton field compared with in the control cotton field in both the Ck poplar and Pb29 poplar ecosystems; the populations dramatically reduced in 2011 compared with in 2009 and 2010, demonstrating the high resistance of Bt toxin towards this target insect species (Table 2). The seasonal dynamics of *S. derogata* Fabricius populations showed the same trend in all 3 years, with peak values in September (Figure 4).

**Table 2.** Population of *Grnsonoma minutara* Hubner and *Sylepta derogata* Fabricius of transgenic poplar-cotton ecosystem (number).

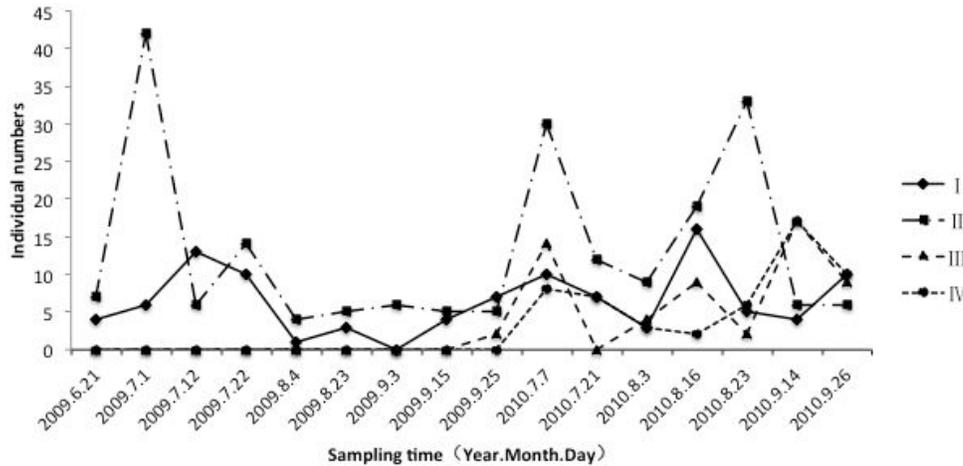
| Year | Sample area                                 | I   | II  | III | IV |
|------|---|-----|-----|-----|----|
| 2009 | Poplar ( <i>Grnsonoma minutara</i> Hubner)  | 48  | 94  | 2   | 0  |
|      | Cotton ( <i>Sylepta derogata</i> Fabricius) | 362 | 4   | 148 | 0  |
| 2010 | Poplar ( <i>Grnsonoma minutara</i> Hubner)  | 55  | 115 | 55  | 53 |
|      | Cotton ( <i>Sylepta derogata</i> Fabricius) | 432 | 0   | 532 | 11 |
| 2011 | Poplar ( <i>Grnsonoma minutara</i> Hubner)  | 7   | 8   | 6   | 3  |
|      | Cotton ( <i>Sylepta derogata</i> Fabricius) | 86  | 0   | 60  | 1  |

I: Ck poplar and Ck cotton; II: Ck poplar and Bt cotton; III: high-resistant Pb29 poplar and Ck cotton; IV: high-resistant Pb29 poplar and Bt cotton.



**Figure 4.** Seasonal dynamics of *Sylepta derogata* Fabricius in the cotton field of transgenic poplar-cotton ecosystem. Ecosystems I-IV are described in Figure 1. Sampling of *S. derogata* Fabricius is described in Material and Methods.

The number of poplar *G. minutara* Hubner was dramatically reduced in the transgenic Pb29 poplar in systems III and IV compared with the control poplar in ecosystems I and II in 2009. The population number in 2010 was comparable between different ecosystems, but decreased in all ecosystems in 2011 compared with those in 2009 and 2010 (Table 2). As shown in Figure 5, the seasonal dynamics of *G. minutara* Hubner showed earlier outburst in ecosystems I and II compared with in ecosystems III and IV, showing remarkable inhibitory effects of the Bt toxin from high-resistant strain Pb29 on this insect species.



**Figure 5.** Seasonal dynamics of *Grnsonoma minutara* Hubner in the poplar field of transgenic poplar-cotton ecosystem. Ecosystems I-IV are described in Figure 1. Sampling of *G. minutara* Hubner is described in Material and Methods.

### Effect of transgenic poplar-cotton ecosystem on the population of non-target aphids *C. po-pulialbae* and *A. gossypii*

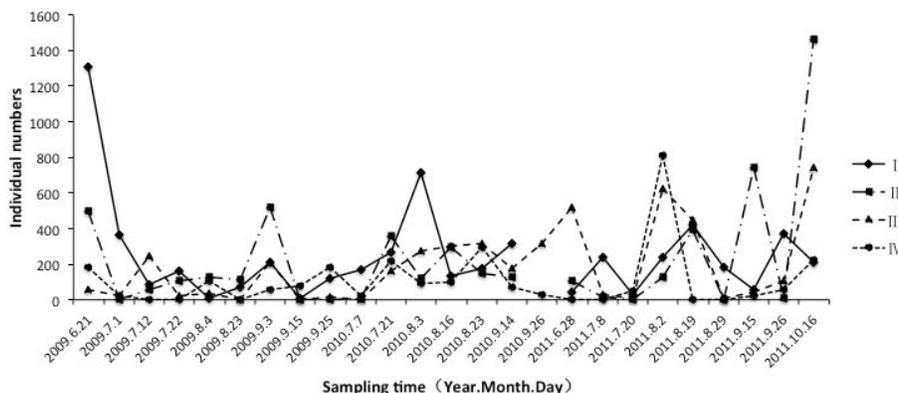
We then investigated non-target effects of ecosystem I-IV on *C. po-pulialbae* in poplar field and *A. gossypii* in cotton field. Our data demonstrated that both cotton and poplar fields were severely inhabited by the 2 types of non-target insects compared to the other target insects tested. The *C. po-pulialbae* population was relatively lower in ecosystems III and IV than in ecosystems I and II, although in varying degrees in different years, indicating the negative impacts of Pb29 poplar on *C. po-pulialbae*. Moreover, the population of *C. po-pulialbae* was lower in ecosystem IV than in ecosystem III in 2010 and 2011, illustrating that the negative impact of transgenic poplar on *C. po-pulialbae* was enhanced by Bt cotton in the same ecosystem (Table 3). The seasonal dynamics of the *C. po-pulialbae* population showed the same trend (Figure 6).

**Table 3.** *Chaitophorus po-pulialbae* population of transgenic poplar-cotton ecosystem (number).

| Year | Sample area | I    | II   | III  | IV   |
|------|-------------|------|------|------|------|
| 2009 | Poplar      | 2336 | 1418 | 608  | 626  |
| 2010 | Poplar      | 1770 | 1058 | 1546 | 822  |
| 2011 | Poplar      | 1797 | 2850 | 2521 | 1165 |

I: Ck poplar and Ck cotton; II: Ck poplar and Bt cotton; III: high-resistant Pb29 poplar and Ck cotton; IV: high-resistant Pb29 poplar and Bt cotton.

In contrast to *C. po-pulialbae*, transgenic Bt cotton did not show obvious negative impacts on non-target *A. gossypii* populations in the transgenic poplar or Ck poplar ecosystem, as indicated by total number of insects and seasonal dynamic data (Table 4 and Figure 7).

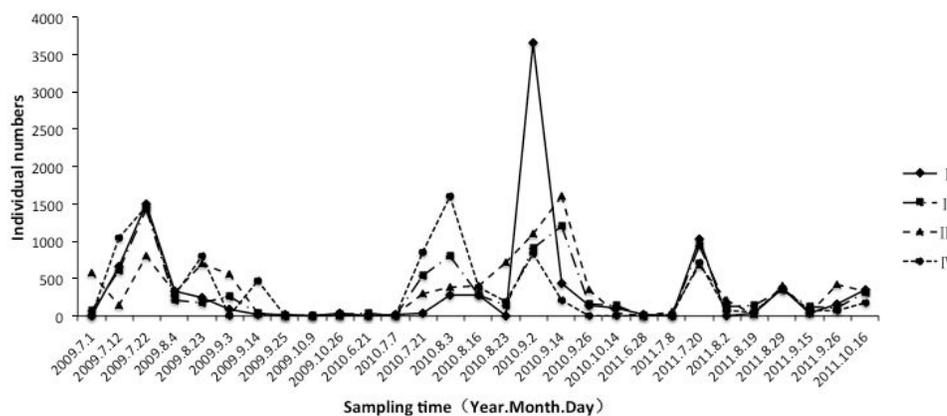


**Figure 6.** Seasonal dynamics of *Chaitophorus po-pulialbae* in the cotton field of transgenic poplar-cotton ecosystem. Ecosystems I-IV are described in Figure 1. Sampling of *C. po-pulialbae* are described in Material and Methods.

**Table 4.** *Aphis gossypii* population of transgenic poplar-cotton ecosystem (number).

| Year | Sample area | I    | II   | III  | IV   |
|------|-------------|------|------|------|------|
| 2009 | Cotton      | 2905 | 2823 | 3127 | 4063 |
| 2010 | Cotton      | 4957 | 4199 | 4916 | 4097 |
| 2011 | Cotton      | 1985 | 2114 | 2163 | 1542 |

I: Ck poplar and Ck cotton; II: Ck poplar and Bt cotton; III: high-resistant Pb29 poplar and Ck cotton; IV: high-resistant Pb29 poplar and Bt cotton.



**Figure 7.** Seasonal dynamics of *Aphis gossypii* in the cotton field of transgenic poplar-cotton ecosystem. Ecosystems I-IV are described in Figure 1. Sampling of *A. gossypii* are described in Material and Methods.

**Effect of transgenic poplar-cotton ecosystem on the population of target insect *H. armigera***

As shown in Table 5, infestation of cotton fields with *H. armigera* in the 2009-2011 seasonal years was low, and the transgenic Bt cotton showed significant inhibitory effects on

its target insect *H. armigera* whether the Bt cotton was in the Ck poplar or Pb29 poplar ecosystems.

**Table 5.** *Heliothis armigera* population of transgenic poplar-cotton ecosystem (number).

| Year | Sample area | I | II | III | IV |
|------|-------------|---|----|-----|----|
| 2009 | Cotton      | 8 | 0  | 10  | 1  |
| 2010 | Cotton      | 3 | 1  | 12  | 0  |
| 2011 | Cotton      | 6 | 0  | 6   | 0  |

I: Ck poplar and Ck cotton; II: Ck poplar and Bt cotton; III: high-resistant Pb29 poplar and Ck cotton; IV: high-resistant Pb29 poplar and Bt cotton.

## DISCUSSION

Crop biotechnology primarily refers to the technology for generating genetically modified crops and has become a rapidly developing field in modern agriculture. Since the introduction of Bt transgenic crops for commercial application, there has been concern regarding their efficacy and impacts on both target and non-target insects. Numerous field studies evaluating the effects of transgenic crops on insect pests to date have focused on the diversity and abundance of insect species in mono-crop conditions, while relatively few studies have examined these effects in complex agro-ecosystems. The poplar-cotton intercrop system is the main agricultural planting mode in plain cotton regions of China. With the development of transgenic cotton and transgenic poplar, it is important to investigate the effects of this transgenic system on insect species. In a field study conducted over 3 years, the population dynamics and seasonal densities of several target and non-target insect species were evaluated in 4 different agro-ecosystems. Lepidopteran caterpillars are the target pests of Bt toxin. Populations of target insects Fall webworm moth, *G. minutata* Hubner, *S. derogata* Fabricius, and *H. armigera* were investigated; the results demonstrated that transgenic Bt cotton in combination with transgenic Pb29 poplar showed higher inhibitory effects on Fall webworm moth, illustrating synergistic effects of transgene expression on this insect species. This is the first study showing intercrop combinatory effects on a target insect species. Another important finding was that Pb29 transgenic poplar inhibited the growth of a non-target hemipteran insect *C. pulialbae*, a sucking insect, while the *A. gossypii* population remained unaffected on Bt cotton. Some studies have reported that Bt toxin was not transported in the phloem (Head et al., 2001; Raps et al., 2001). Hemipteran sucking insects feed on phloem sap, so it is reasonable to speculate that the Bt toxin does not interfere with sucking insects. However, some others have demonstrated the effectiveness of the Bt toxin against *Macrosiphum euphorbiae* (Homoptera: Aphididae) and *Nilaparvata lugens* (Homoptera: Delphacidae) in transgenic potato and rice, respectively (Ashouri et al., 2001; Chen et al., 2003a,b). Furthermore, Hunt et al. (2006) reported that expression of Bt toxin may affect sap-feeding aphids by influencing phloem sap composition. The structure and phloem sap composition of cotton and poplar may be quite different, and therefore had different effects on exogenous Bt expression, leading to varying effects of these crops on sucking insects. Interestingly, the negative effect of Pb29 transgenic poplars on the population of the non-target insect *C. pulialbae* was enhanced by transgenic Bt cotton in the same agro-ecosystem. Thus, the transgenic cotton-poplar ecosystem may have species-specific synergistic effects on both target and non-target species, although the mecha-

nism underlying this phenomenon requires further investigation.

The impacts of Bt transgenic cotton on a variety of insect species were investigated by randomly selecting Bt transgenic poplars (Zhang et al., 2011). Therefore, our data provide insight into the effects of transgenic Bt toxin on both target and non-target insects in a poplar-cotton intercrop system from an agro-ecological perspective.

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