Transgenic Res (2011) 20:523–532
DOI 10.1007/s11248-010-9434-1

ORIGINAL PAPER

Expression of Bt-Cry3A in transgenic *Populus alba* × *P. glandulosa* and its effects on target and non-target pests and the arthropod community

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Received: 8 May 2010 / Accepted: 27 July 2010 / Published online: 12 August 2010

Abstract During the growing seasons of 2006–2008, feeding tests and field studies were conducted in Beijing, China, to investigate the effects of transgenic *Bacillus thuringiensis* (Bt) poplar (BGA-5) expressing the Cry3A protein (0.0264–0.0326% of the total soluble protein) on target and non-target pests and the arthropod community. The effects of BGA-5 on the target pest *Plagiodera versicolora* (Coleoptera, Chrysomelidae) and a non-target pest *Clostera anachoreta* (Lepidoptera, Notodontidae), were assessed under laboratory conditions. Total mortality of *P. versicolora* larvae fed with BGA-5 leaves was significantly higher than that of the control ($P < 0.05$). The exuviation index of *P. versicolora* larvae fed with BGA-5 tended to be higher than that of CK, but it was not significantly different. The pupation rate and eclosion rate of the survived larvae fed with BGA-5 were lower than that of CK, but it was also not significantly different. Additionally, no significant differences were detected in the mortality, exuviations index, pupation rate, or eclosion rate of *C. anachoreta* fed with leaves of transgenic and non-transgenic poplars. Furthermore, the arthropod communities in the Bt poplar and CK field stands were similar, as indicated by four diversity indices (Berge-Parker index, Shannon-Wiener indices, evenness index, and Simpson's inverted index) and the Bray-Curtis index. Therefore, the Bt-Cry3A poplar decreased damage by the target pest (*P. versicolora*), had no effects on a non-target pest (*C. anachoreta*), and generally did not have any significant negative effect on the poplar arthropod community.

Keywords *Bacillus thuringiensis* · Cry3A expression · Insect bioassay · Transgenic poplar · Arthropod community

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Introduction

Poplar (*Populus* L.) is an important forest genus worldwide. Commercial and ecological poplar plantations have expanded rapidly in recent years to meet the greatly increased demands for fiber, timber, and fuel. In China, poplar plantations covered nearly 7 mha in 2008 (Lu 2008). However, pest and disease damage has become a serious problem. Forest insects and diseases have damaged large areas in China, with 8270,000 ha totally lost to insect and disease damage,

870,000 ha affected by poplar defoliating insects, and 710,000 ha occupied by poplar stem borers in 2005 (Zhang and Liu 2006).

Deploying genetically engineered insect-resistant trees is a modern alternative to chemical insecticides for tree pest management. In poplars, various insect-resistant genes have been transferred to genetically engineered trees for pest control since 1991 (McCown et al. 1991; Leplé et al. 1995; Kang et al. 1997; Confalonieri et al. 1997, 1998; Delledonne et al. 1998; Hao et al. 1999; Wu et al. 2000; Delledonne et al. 2001; Wang and Constabel 2004). Cry genes (or modified Cry genes), originally from the spore forming bacterium *Bacillus thuringiensis* (Bt) and encoding the insecticidal crystal protein, are commonly used to produce genetically engineered crops and trees with insect-resistant traits. Cry proteins are highly specific and generally only individual insect orders such as Lepidoptera, Coleoptera, and Diptera are susceptible (Schnepf et al. 1998). Two transgenic lepidopteron-resistant poplar clones have been commercialized in China since 2002. One is Bt *Populus nigra* and the other is transgenic hybrid poplar 741 (*P. alba* L. × (*P. davidiana* Dode + *P. simonii* Carr. × *P. tomentosa* Cart.) containing both *Bt-Cry1AC* and the arrowhead proteinase inhibitor gene (*API*) (Hu et al. 2001; Zheng et al. 2000). Bt *P. nigra* was the first Bt-transgenic tree to be commercialized and covered 500–600 ha China by 2005 (Ewald 2005). After several years of cultivation, studies have shown that transplanting transgenic insect-resistant poplars is an effective method for controlling the large-scale pest damage caused by some key target pests on poplar plantations (Hu et al. 2001, 2007; Gao et al. 2003).

Although there are many concerns in regard to risks of transgenic poplar to forest ecosystems because of these trees' long life cycles and complex interactions with other organisms (James et al. 1998), related reports are very few due to the restrictions on environmental release of transgenic tree in many countries. China is the first and only country which commercialized the Bt transgenic poplars, and now it has the largest transgenic poplar plantations in the world, therefore the possibility of ecological risk associated with these plantations has attracted much attention. Several previous studies have examined the two commercialized transgenic poplars, primarily to assess their impact on non-target insect, natural enemy, and arthropod communities. Yao et al. (2006) investigated *Harmonia axyridis* (Pallas), a predator of *Chaitophorus populeti* (Panzer), but found no significant difference in predator survival, body mass, eclosion, or sex ratio when *C. populeti* were collected from field-grown transgenic hybrid 741 and the corresponding control. They also found that the pupal duration of these ladybeetles was lower than the control, but the reason remained unknown. Jiang et al. (2009) reported that the numbers of the non-target sucking pests *C. populeti* (Panzer) and *Misumenops tricuspidatus* (Fabricius), the predator *H. axyridis* (Palla), and the parasitoid *Vulgichneumon leucaniae* (Uchida) all differed between transgenic hybrid 741 and its control. Zhang et al. (2004) discovered an obvious difference in the dominant insect community species between a pure transgenic Bt *P. nigra* stand and a mixed poplar stand. It was also shown that there was more variety, number, and an increased parasitic ratio of the natural insect enemies in a transgenic Bt *P. nigra* poplar plantation compared to non-transgenic poplar plantations (Hu et al. 2007). The influence of double insect-resistant genes on the arthropod community in transgenic hybrid poplar 741 was also studied with results indicating that this transgenic poplar could effectively decrease the number of defoliating insects, lower the dominance of the dominant species, and increase insect diversity and the evenness of the insect pest sub-community. Meanwhile, the similarity in communities between transgenic and non-transgenic poplars decreased with increasing transgenic poplar insect resistance (Gao et al. 2003). A later report on the arthropod community nutritional structure variation and ecological niche in plantations of transgenic hybrid poplar 741 showed that insect-resistant gene expression in the transgenic poplar influenced the feeding behavior of different insect functional groups (Gao et al. 2006). It is clear from these reports that defoliating insect-resistant transgenic poplars might have some effects on non-target insects and could possibly influence insect community structure.

To prevent tree damage by coleopterous insects, transgenic *P. alba* × *P. glandulosa* cv'84 k' with the coleopterous specific gene (Bt-Cry3A, under the CaMV35S promoter, and NPTII as selection marker) were produced. Bioassay with stems of 1-year-old transgenic and non-transgenic poplars showed that

one transgenic clone, referred to as to BGA-5 poplar, was toxic to *Anoplophora glabripennis* (Motschulsky)(Coleoptera, Cerambycidae) larvae with 30% mortality and a 78.46% growth inhibition rate (Zhang et al. 2006). BGA-5 was permitted for use in a field trial, and a transgenic plantation was established in 2005. The aim of this study was to estimate coleopterous insect resistance of transgenic poplar using *Plagiodera versicolora* (Laicharting), a leaf beetle (Coleoptera, Chrysomelidae) that damages young poplar leaves (Yang et al. 2006) and to test its effects on the non-target pest *Clostera anachoreta* (Fabricius) (Lepidoptera, Notodontidae). The composition structure and community characteristics of the arthropod community were monitored in a field trial to unravel the effects on the arthropod community because it is unknown if the coleopterous insect-resistant transgenic poplar could influence insect community structure after being released into the natural environment.

Regulatory approval

Field trial approval of BGA-5 poplar was obtained from the State Forestry Administration, People's Republic of China, under application number 2005-05.

Materials and methods

Field trial and plant material

The BGA-5 field trial and the corresponding non-transgenic wild-type *P. alba* × *P. glandulosa* cv'84 k' as a control (wt control, referred as to CK) were established in 2005 in Beijing, China. Trees were planted in lines of 100 trees each (10 rows and 10 columns) in a field with 2.0 m intervals between trees. All trees were greenhouse-grown cuttings of BGA-5 and CK. Except for irrigating the plantlets at planting time, no irrigation, supplemental fertilization, pesticides, or herbicides were used.

ELISA analysis of Bt-Cry3A concentration in BGA-5 leaves

Fully expanded leaves (7th–9th leaves in branches) were collected from five randomly selected field-grown BGA-5 trees and five non-transgenic CK poplar trees in July 2006, 2007, and 2008. The samples were kept on ice and were immediately weighed to the nearest 0.5 g in the laboratory to determine the Bt-Cry3A protein levels. The Bt-Cry3A protein level was determined by enzyme-linked immunosorbent assays (ELISA) using a Bt-Cry3A ELISA kit (Agdia Inc., Elkhart, IN, USA) following the manufacturer's procedure. The total leaf protein level was determined by staining with Coomassie blue.

Effects on the BGA-5 target pest

The *P. versicolora* egg masses were collected from poplar trees near the Chinese Academy of Forestry and hatched under laboratory conditions at 28–32°C. Healthy 1-day-old larvae were used for the feeding experiment. The larvae were fed fresh, clean, fully expanded leaves collected from BGA-5 and CK trees every day. There were 20 larvae per treatment (confined together), and the experiment was repeated three times. Dead larvae were removed and recorded each day. Larval exuviation was observed 3 and 5 days later. The following equations were used:

Total mortality rate = (number of dead larvae at the end of feeding/total number of larvae) × 100%

Exuviation index = (first instar × larvae number + second instar × larvae number + ⋯ + last instar × larvae number)/total number of larvae × 100%

Pupation rate = (pupae number/larvae number) × 100%

Eclosion rate = (progeny emerged/total number of pupae) × 100%

Effects on the BGA-5 non-target pest

Clostera anachoreta egg masses were collected from poplar trees near the Chinese Academy of Forestry and hatched under laboratory conditions at 28–32°C. Healthy 1-day-old larvae were used in the feeding experiment. The larvae fed fresh, clean, transgenic and non-transgenic poplar leaves every day. There were 20 larvae per treatment (confined together), and the experiment was repeated three times. Dead larvae were removed and recorded each day. The rate of total mortality, larval exuviation, pupation, and eclosion was calculated using the above equations, as described above.

Monitoring composition structure and arthropod community characteristics in the field trial

The insect fauna in the transgenic and non-transgenic poplars were sampled 16 times during the growing seasons (from April to October) of 2006, 2007, and 2008 (4 times in 2006, 5 times in 2007, and 7 times in 2008). The trees were chosen by a random sampling method, and 20 trees per line were sampled.

Insects appearing on the leaves, branches, and bark of each tree were observed and identified. The species and number of arthropods were recorded for each tree. The insects that could not be classified in the field were preserved in alcohol and identified later with a microscope in the laboratory.

The arthropod community in the poplar plantation was classified into four guilds based on nutritional relationships, i.e., phytophages, parasitoids, predators, and others, referring to Heong et al. (1991). The composition and number of arthropod guilds and common community indices were compared between the transgenic and non-transgenic poplars, including the species richness (S), Shannon-Wiener index $\left(H', H' = -\sum_{i=1}^{s} Pi \ln Pi\right)$, evenness index ($J$, $J = H'/\ln S$), Berge-Parker index (I, $I = Ni/N$), and Simpson's inverted index $\left(1/D, 1/D = \sum_{i=1}^{s} \frac{Ni(Ni-1)}{N(N-1)}\right)$. All were defined and calculated as by Magurran (1988). The Bray-Curtis (BC) dissimilarity index was calculated to measure the dissimilarity in the arthropod community between the transgenic (j) and non-transgenic (k) poplars. The BC index is a measure of dissimilarity between two sample sets and ranges from 0 (similar) to 1 (dissimilar) (Krebs 1989):

$$\mathrm{BC} = \frac{\sum_{i=1}^{n} |x_{ij} - x_{ik}|}{\sum_{i=1}^{n} |x_{ij} + x_{ik}|}$$

where x_{ij} and x_{ik} are the number of individuals in the ith family of the transgenic (j) and non-transgenic (k) poplars.

Statistical analysis

The statistical package SPSS 16.0 (SPSS Inc., IL, USA) for windows was used for all statistics. One-Way ANOVA was used to compare mortality rate, exuvation index, pupation and eclosion rates in feeding experiments and Bt-Cry3A protein content in leaves. Percentage data were arcsin transformed before the analysis of variance. Origin8.0 SR2 (OriginLab Corp., Northampton, MA, USA) for Windows was used for graphs.

Results

Concentration of Bt-Cry3A toxin in transgenic poplar leaves and its toxicity to the phytophagous beetle *P. versicolora*

Quantification of the Bt-Cry3A protein by ELISA indicated that the mature leaves of BGA-5 poplar contained 13,557 ± 508 (mean ± S.E.) ng/g, 12,968 ± 386 ng/g, and 13,005 ± 339 ng/g of Bt-Cry3A protein in mid-July of 2006, 2007, and 2008, respectively, which corresponded to 0.0264–0.0326% of the total soluble protein. The Bt-Cry3A level did not significantly differ between the 3 years ($F = 0.626$, $df = 2$, $P = 0.551$). No Bt-Cry3A protein was detected in the non-transgenic CK leaves.

One-day-old *P. versicolora* larvae were fed with transgenic BGA-5 or non-transgenic poplar leaves each day, and the mortality was recorded. The total mortality of *P. versicolora* larvae fed with BGA-5 leaves was 70.00%, which was significantly higher than the 35.00% of CK ($P < 0.05$) (Table 1).

The periodic exuviation index, pupation, and eclosion rate of *P. versicolora* were calculated to study the influence of BGA-5 on surviving larvae (Table 1). The results showed that the exuviation index of *P. versicolora* larvae fed with BGA-5 was 1.57 on days 3 and 2.60 on days 5, which tended to be higher than that of CK, but it was not significantly different. Both the pupation rate and eclosion rate of the survived larvae fed with BGA-5 were lower than that of CK, but it was also not significantly different.

Effects of the BGA-5 Bt transgenic line on the non-target insect

Larvae of *C. anachoreta* were fed with fresh BGA-5 leaves in the laboratory to study the effect on this non-target insect. As shown in Table 2, there was no significant difference in total larval mortality between BGA-5 and CK.

The exuviation of *C. anachoreta* larvae was observed on days 6 and 12, and the exuviation index

Table 1 Mortality, exuviation index, pupation, and eclosion rate of *Plagiodera versicolora* fed with leaves of the transgenic *Bacillus thuringiensis* (Bt) poplar (BGA-5) expressing the Cry3A protein or the corresponding untransformed line (CK)

Line	Total mortality rate (%)	Exuviation index		Pupation rate (%)	Eclosion rate (%)
		Day 3	Day 5		
CK	35.00 ± 1.12[a]	1.54 ± 0.01[a]	2.45 ± 0.03[a]	100.00 ± 0.00[a]	77.22 ± 0.69[a]
BGA-5	70.00 ± 1.29[b]	1.57 ± 0.01[a]	2.60 ± 0.03[a]	88.89 ± 2.48[a]	70.83 ± 3.36[a]

* Data are mean ± SE (the standard error of the mean)

** Means with the same letter are not significantly different at $P < 0.05$ by the One-Way ANOVA

*** Data are arcsin transformed before the analysis of variance

was calculated to determine if larval development was influenced by feeding on the transgenic leaves of BGA-5. The exuviations index of larvae fed BGA-5 on days 6 and days 12 tended to be lower than that for CK, but the differences were not significant both on days 6 and 12 (Table 2).

Clostera anachoreta pupation and eclosion rates were also calculated for surviving larvae. The pupation rate of larvae fed on BGA-5 was 76.67%, and the eclosion rate of adults fed BGA-5 was 82.44%. There was no significant difference in either the pupation rate or eclosion rate between BGA-5 and CK (Table 2).

Arthropod composition and family dominance

The Bt-Cry3A protein in the transgenic BGA-5 poplar is toxic to coleopterous insects, and thus the number of coleopterous insects was expected to be lower in the transgenic poplar than in CK in the field trial. During the 3-year study, 4956 arthropod individuals were observed in the field trial, with 2552 on the non-transgenic control *P. alba* × *P.glandulosa* cv'84 k' trees and 2,404 on transgenic BGA-5, belonging to 10 orders and 41 families. The arthropods included four functional guilds, i.e., phytophages, predators, parasitoids, and others. There were 37 families on CK, but the Lasiocampidae, Aegeriidae, Coreoidea, and Miridae families were absent compared to those on BGA-5. Although the families and individuals in the functional arthropod groups were not the same, the dominant families in each guild were similar. The most abundant families were Aphidiae and Gracillariidae in phytophages, Formicidae and Coccinellidae in predators, and Braconidae and Ichneumonidae in parasitoids (Fig. 1).

The phytophages of the arthropod community were classified into three classes based on food eating habits and insect resistance to the transgenic poplar, i.e., targeted pests of Coleoptera, non-targeted foliage-eating pests, and sucking pests. The dominance of targeted insects was lower on BGA-5 than on CK, showing that the Bt-transgenic poplar retained coleopteran insect-resistant ability in the field. The dominance of non-targeted insects also decreased on BGA-5 transgenic poplar, mainly due to the decreased number of Notodontidae [*Clostera anachoreta* (Fabricius) and Gracillariidae (*Phyllonorycter populiella*

Table 2 Mortality, exuviation index, pupation, and eclosion rate of *Clostera anachoreta* fed with leaves from the Bt-Cry3A transgenic poplar (BGA-5) or leaves from the corresponding untransformed line (CK)

Line	Total mortality (%)	Exuviation index		Pupation rate (%)	Eclosion rate (%)
		Day 6	Day 12		
CK	21.67 ± 3.59[a]	2.63 ± 0.02[a]	4.36 ± 0.02[a]	75.00 ± 1.71[a]	76.50 ± 1.90[a]
BGA-5	23.33 ± 0.75[a]	2.27 ± 0.02[a]	4.29 ± 0.03[a]	76.67 ± 0.75[a]	82.44 ± 1.29[a]

* Data are mean ± SE (the standard error of the mean)

** Means with the same letter are not significantly different at $P < 0.05$ by the One-Way ANOVA

*** Percent data are arcsin transformed before the analysis of variance

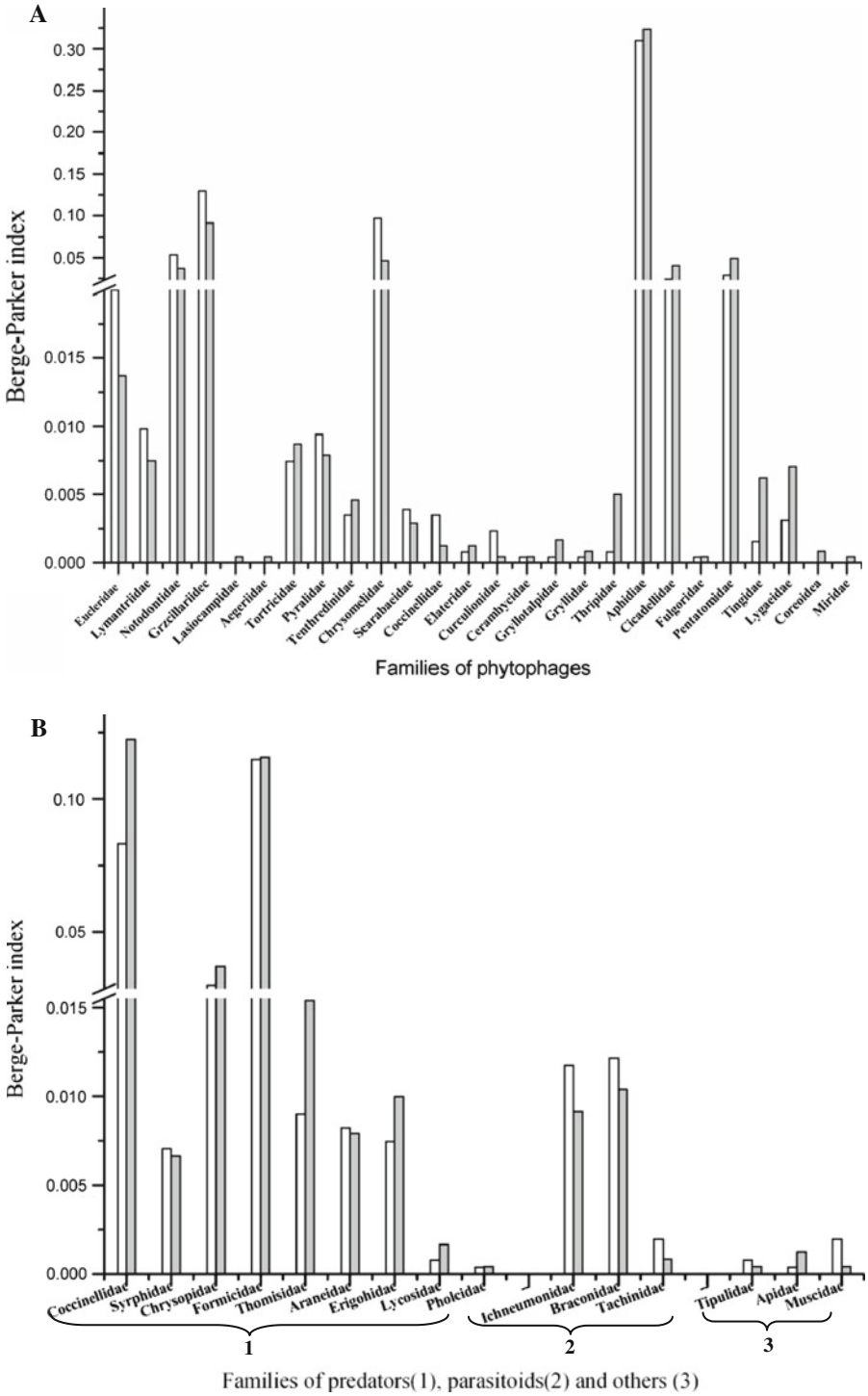

Fig. 1 Family composition of the arthropod community and their relative dominance in transgenic *Bacillus thuringiensis* (Bt) poplar (BGA-5, *gray column*) expressing the Cry3A protein and in the corresponding untransformed line (CK, *white column*). **a** Family composition in guild of phytophages. **b** Family composition in guilds of parasitoids, predators and others

(Zeller)] insects. But the dominance of sucking pests increased on BGA-5 due to the increased number of minor sucking families, such as Cicadellidae [*Empoasca flavescens* (Fabricius)], Pentatomidae [*Eurydema dominulus* (Scopoli)], and Tingidae [*Hegesidemus habrus* (Darke)] (Table 3, Fig. 1). This indicated that the Bt-transgenic BGA-5 was able to prevent an increase in the number of targeted insects.

Table 3 The Berge-Parker dominance index of different insect class dominance in the arthropod community of transgenic (BGA-5) and non-transgenic *Populus abla* × *P. glandulosa* (CK)

Line	Targeted insects	Non-targeted defoliator	Sucking pest
CK	0.1078	0.2343	0.3683
BGA-5	0.0528	0.1743	0.4334

Arthropod community guild analysis

The Berge-Parker index, Shannon-Wiener index, evenness index, and Simpson's inverted index showed no large difference between Bt-transgenic BGA-5 and CK in the entire arthropod community and in each of the four guilds (Table 4). The Bray-Curtis (BC) dissimilarity indices between BGA-5 and CK were 0.1403 in phytophages, 0.0946 in predators, 0.1478 in parasitoids, 0.5385 in others, and 0.1283 in the entire community. Except for the "other" guild, the dissimilarity indices were low, indicating that the guilds and communities were similar for BGA-5 and CK. The BC index between the "other" guild was high, mainly due to low numbers and low diversity in that guild.

Discussion

The ELISA analysis showed that the Bt-Cry3A protein was stably expressed in the BGA-5 transgenic poplar line during a 3-year field trial. The Bt-Cry3A protein in field-grown BGA-5 leaves did have some lethal effects on *P. versicolora* larvae in a laboratory feeding experiment, which was reflected in the higher mortality of *P. versicolora* larvae compared to CK. But the development of the survived *P. versicolora* larvae was not be influenced by feeding the leaves containing Bt-Cry3A protein, which suggested that this toxin in transgenic poplar did not have similar sublethal effects to this target insect as it did to another target insect *A. glabripennis* in our previous study (Zhang et al. 2006). This is not good for controlling the damage caused by *P. versicolora* because this pest usually has 6–8 generations per year in many areas of China (Yang et al. 2006). In the field observations, we observed that the dominance of coleopteran insects was lower on BGA-5 than CK, suggesting that the Bt-transgenic poplar could decrease the amount of this multiple-generation target pest under field conditions. The development rate of a pest's resistance is positively correlated with increasing selection pressure (Tabashnik et al. 1990) and Bt-Cry3A-resistant target beetle strains have been reported in some studies under prolonged exposure (Whalon et al. 1993; Augustin et al. 2004). Although BGA-5 did not have high selection pressure on *A. glabripennis* and *P. versicolora*, further studies are needed to determine if these

Table 4 Main indices of arthropod community diversity in Bt-Cry3A transgenic (BGA-5) and non-transgenic *Populus. abla* × *P. glandulosa* (CK)

Guild	Indices	Line	
		CK	BGA-5
Phytophages	*S*	22	26
	I	0.7104	0.6606
	H′	1.8164	1.8497
	J	0.5876	0.5677
	1/*D*	0.2517	0.2774
Predators	*S*	9	9
	I	0.2606	0.3170
	H′	1.4255	1.4513
	J	0.6488	0.6605
	1/*D*	0.3115	0.2991
Parasitoids	*S*	3	3
	I	0.0259	0.0204
	H′	0.9088	0.8334
	J	0.8272	0.7586
	1/D	0.4242	0.4524
Others	*S*	3	3
	I	0.0031	0.0021
	H′	0.9003	0.9503
	J	0.8194	0.8650
	1/*D*	0.1484	0.1512
Arthropod	*S*	37	41
	I	0.3100	0.3236
	H′	2.3941	2.4312
	J	0.6630	0.6547
	1/*D*	0.1484	0.1512

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S species richness, *I* Berge-Parker index, *H*′ Shannon-Wiener index, *J* evenness index, 1/*D* Simpson's inverted index

insects develop resistance after long-term feeding on poplar leaves containing the Bt-Cry3A toxin.

In our previous laboratory feeding experiment, this line only produced 30% mortality in *A. glabripennis* larvae (Zhang et al. 2006). Compared to Bt-Cry3A potato (5.71 ± 1.06 ng/mg) and Bt-Cry3A hybrid poplars (0.05–0.0025% of total soluble protein), the amount of Bt-Cry3A toxin of BGA-5 is not low (approximately 2.97–1.36 ng/mg, 0.0264–0.0326% of the total soluble protein), but its mortality to target insects was very low (Cooper et al. 2004; Génissel et al. 2003). Another transgenic hybrid 741 poplar containing the same Bt-Cry3A also had low lethality to *Apriona germari* (Hope) (Coleoptera, Cerambycidae). Therefore *A. glabripennis*, *P. versicolora* and *A. germari* are inherently less susceptible to Bt-Cry3A. A modified Cry3A has greatly increased toxicity to chrysomelid beetle of the genus Diabrotica (US Patent no. 7,030,295). The Bt-Cry3A gene used in our study needs to be modified according to the gut proteases and toxin binding properties of the target insects.

In general, cry proteins are highly specific and are often only toxic to individual insect orders (Van Frankenhuyzen 1993). The Bt δ-endotoxins Cry3A in our BGA-5 transgenic line and Cry1B exhibit coleopteran specific activity (McPherson et al. 1988; Bradley et al. 1995). However, potential risks to non-target insects must be studied to assess environmental safety. *Clostera anachoreta* (Fabricius) (Lepidoptera, Notodontidae) is a major and widespread poplar leaf eating pest in China and is a good candidate for testing the non-target effects of the Bt transgenic poplar (Liu et al. 2009). Our laboratory feeding results on *C. anachoreta* showed that the BGA-5 Bt-Cry3A transgenic line had no toxic effect on this non-target insect. Current knowledge of the insecticidal activity of this toxin indicates limited toxicity against other insect groups (Raybould et al. 2007). Although further multiple-generation feeding experiments and long-term field trials are needed, it is unlikely that the Bt poplar is toxic to non-target insects.

In general, the decrease in target insects on transgenic plants will benefit other defoliators and lead to an increase in some non-target pests, as observed for Bt poplar, rice, and cotton (Fitt et al. 1994; Cui and Xia 1998; Liu et al. 2002, 2003; Zhang et al. 2004). The sucking pests on BGA-5 did increase, but the numbers of other main non-targeted defoliators such as *C. anachoreta* and *Phyllonorycter populiella* (Zeller) on BGA-5 were lower than on CK. This was unlikely to be due to the toxicity of the Bt toxin in the transgenic poplar from our laboratory feeding experiment on non-target *C. anachoreta* larvae. Some species such as *Harmonia axyridis* (Pallas) and *Propylea japonica* (Thunberg) in Coccinellidae are natural enemies of *C. anachoreta* and *P. populiella* (Zeller). We found that the number of coccinellid insects increased on BGA-5. Therefore, one possible reason for the decrease in the main non-targeted defoliators might be that the increased number of sucking pests provided food to predators such as coccinellid insects, and that the increased number of predators controlled the non-targeted defoliators. The ecological system in a poplar plantation is very complex, and further study is needed to explore the true reason for the decreased numbers of non-targeted defoliators on Bt transgenic poplar.

Although there were some differences in guild dominance, family composition, and dominance, the Bt poplar and the CK arthropod communities were similar as shown by the four diversity indices and the BC index. These results suggest that planting Bt-Cry3A poplar generally did not have any significant negative effect on the poplar arthropod community.

Due to field trial approval demands (State Forestry Administration, People's Republic of China), the field trial time was short, and the area for our transgenic poplar was small, so mobile insects might have moved between the transgenic plot and the CK plot, which could have caused some errors in our field investigation data. Therefore, another field trial in a large area with a longer investigation time is needed to confirm the present results.

Acknowledgments We appreciate the assistance of Dr. Gao Hai-bo, College of Biological Science and Technology, Beijing Forestry University, for his help with the statistical analysis. This work was supported by the National High-Tech R&D Program (2009AA10Z107) and the Key Program of Genetic Engineering of New organisms of China (2009ZX08009-098B).

References

Augustin S, Courtin C, Rejasse A, Lorme P, Genissel A, Bourguet D (2004) Genetics of resistance to transgenic *Bacillus thuringiensis* poplars in *Chrysomela tremulae*

(Coleoptera: Chrysomelidae). J Econ Entomol 97(3): 1058–1064

Bradley D, Harkey MA, Kim MK, Biever KD, Bauer LS (1995) The insectidal CryIB crystal protein of *Bacillus thuringiensis* ssp. thuringiensis has dual specificity to Coleopteran and Lepidopteran larvae. J Invertebr Pathol 65:162–173

Confalonieri M, Allegro G, Delledonne M (1997) Transgenic black poplars expressing different soybean protease inhibitors genes. In: Abstracts of joint meeting of the IUFRO working parties "Somatic cell genetics and molecular genetics of trees", Québec City, 12–16 August 1997, pp 4–7 and 4–6

Confalonieri M, Allegro G, Balestrazzi A, Fogher C, Delledonne M (1998) Regeneration of *Populus nigra* transgenic plants expressing a Kunitz proteinase inhibitor (KTi3) gene. Mol Breed 4:137–145

Cooper SG, Douches DS, Grafius EJ (2004) Combining genetic engineering and traditional breeding to provide elevated resistance in potatoes to Colorado potato beetle. Entomol Exp Appl 112:37–46

Cui JJ, Xia JY (1998) Effects of transgenic Bt cotton (with early maturity) on population dynamics of main pests and their natural enemies. Acta Gossypii Sin 10(5):255–262

Delledonne M, Belenghi B, Confalonieri M (1998) Genetic transformation of white poplar with different cysteine proteinase inhibitor genes. In: Abstracts of the IXth international congress of plant tissue and cell culture, Jerusalem, 14–19 June 1998

Delledonne M, Allegro G, Belenghi B, Balestrazzi A, Picco F, Levine A, Zelasco S, Calligari P, Confalonieri M (2001) Transformation of white poplar (*Populus alba* L.) with a novel *Arabidopsis thaliana* cysteine proteinase inhibitor gene and analysis of insect pest resistance. Mol Breed 7:35–42

Ewald D (2005) Seeing once is better than studying a thousand times. http://www.gmo-safety.eu/en/wood/poplar/325.docu.html. Accessed 6 July 2005

Fitt GP, Mares CL, Llewellyn DJ (1994) Field evaluation and potential ecological impact of transgenic cottons in Australia. Biocontrol Sci Technol 4:535–548

Gao B, Zhang F, Hou D, Wu B, Zhang S, Zhao X (2003) Structure of arthropod community in stands of transgenic hybrid poplar 741. J Beijing For Univ 25(1):62–64

Gao B, Gao S, Liu J, Jiang W (2006) Variation of nutritional structure and ecological niche of arthropod community in plantation of transgenic insect-resistance hybrid poplar 741. Acta Ecol Sin 26(10):3499–3507

Génissel A, Leplé JC, Millet N, Augustin S, Jouanin L, Pilate G (2003) High tolerance against *Chrysomela tremulae* of transgenic poplar plants expressing a synthetic *cry3Aa* gene from *Bacillus thuringiensis* ssp. *tenebrionis*. Mol Breed 11(2):103–110

Hao GX, Zhu Z, Zhu ZT (1999) Transformation of *Populus tomentosa* with insecticidal Cowpea proteinase inhibitor gene. Acta Bot Sin 41:1276–1282

Heong KL, Aquino GB, Barrion AT (1991) Arthropod community structures of rice ecosystems in the Philippines. Bull Entomol Res 81(4):407–416

Hu JJ, Tian YC, Han YF, Li L, Zhang BE (2001) Field evaluation of insect-resistant transgenic *Populus nigra* trees. Euphytica 121:123–127

Hu JJ, Li SM, Lu MZ, Li JX, Li KH, Sun XQ, Zhao ZY (2007) Stability of Insect-resistance of Bt transformed *Populus nigra* plantation and its effects on the natural enemies of insects. For Res 20(5):656–659

James RR, Difazio SP, Brunner AM, Strauss SH (1998) Environmental effects of genetically engineered woody biomass crops. Biomass Bioenergy 14:403–414

Jiang WH, Liu JX, Zhang F, Gao BJ (2009) Population dynamic of target pest, non-target pests and major natural enemy in transgenic hybrid poplar 741. J Shandong Agric Univ (Nat Sci) 40(2):195–199

Kang H, Hall RB, Heuchelin SA, McNabb HS, Mize CW, Hart ER (1997) Transgenic *Populus*: in vitro screening for resistance to cottonwood leaf beetle (Coleoptera: Chrysomelidae). Can J For Res 27(6):943–944

Krebs CJ (1989) Ecological methodology. Harper and Row, New York

Leplé JC, Bonadebottino M, Augustin S, Pilate G, Letan VD, Delplanque A, Cornu D, Jouanin L (1995) Toxicity to *Chrysomela tremulae* (Coleoptera, Chrysomelidae) of transgenic poplars expressing a cysteine proteinase-inhibitor. Mol Breed 1:319–328

Liu WX, Wan FH, Guo JY (2002) Structure and seasonal dynamics of arthropods in transgenic Bt cotton field. Acta Ecol Sin 22:729–735

Liu ZC, Ye GY, Hu C, Datta SK (2003) Impact of transgenic indica rice with a fused gene of crylAb/crylAc on the rice paddy arthropod community. Acta Entomol Sin 46(4): 454–465

Liu J, Gao B, Zhang J, Wang J, Jiang W, Li Y, Zhou G (2004) Sustainability of insect resistance of transgenic hybrid poplar 741. J Beijing For Univ 26(6):76–79

Liu MG, Hou JM, Zhang XH, Han HZ, Zhao YF (2009) Observation on sexual reproduction of *Clostera anachoreta*. For Pest Dis 28(4):1–4

Lu MZ (2008), Advance in transgenic research and its biosafety of forest trees in China. In: Proceeding of the 8th symposium in agricultural biochemistry and molecular biology of the Chinese Society of Biochemistry and Molecular Biology. Guiyang, China, pp 1–2

Magurran AE (1988) Ecological diversity and its measurement. Cambridge University Press, Cambridge

McCown BH, McCabe DE, Russell DR, Robison DJ, Barton KA, Raffa KF (1991) Stable transformation of *Populus* and incorporation of pest resistance by electric discharge particle acceleration. Plant Cell Rep 9:590–594

McPherson S, Perlak F, Fuchs R, Marrone P, Lavrik P, Fischhoff D (1988) Characterization of the Coleopteran-specific protein gene of *Bacillus thuringiensis* var tenebrionis. Biotechnology 6:61–66

Raybould A, Stacey D, Vlachos D, Graser G, Li X, Joseph R (2007) Non-target organism risk assessment of MIR604 maize expressing mCry3A for control of corn rootworm. J Appl Entomol 31(6):391–399

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean DH (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Mol Biol Rev 62:775–806

Tabashnik BE, Cushing NL, Finson N, Johnson MW (1990) Field development of resistance to *Bacillus thuringiensis*

in diamondback moth (Lepidoptera: Plutellidae). J Econ Entomol 83:1671–1676

Van Frankenhuyzen K (1993) The challenge of *Bacillus thuringiensis*. In: Entwistle PF, Cory S, Bailey MJ, Higgs S (eds) *Bacillus Thuringiensis*, an environmental biopesticide: theory and practice. Wiley, Chichester, pp 1–23

Wang J, Constabel CP (2004) Polyphenol oxidase overexpression in transgenic *Populus* enhances resistance to herbivory by forest tent caterpillar (*Malacosoma disstria*). Planta 220:87–96

Whalon ME, Miller DL, Hollingsworth RM, Grafius EJ, Miller JR (1993) Selection of Colorado Potato Beetle (Coleoptera: Chrysomelidae) strain resistance to *Bacillus thuringiensis*. J Econ Entomol 86:226–233

Wu NF, Sun Q, Yao B, Fan YL, Rao HY, Huang MR, Wang MX (2000) Insect-resistant transgenic poplar expressing AaIT gene. Chin J Biotechnol 16:129–133

Yang ZD, Tian XQ, Zhao BG (2006) Threshold and effective accumulative temperature for the development of *Plagiodera versicolora*. J Beijing For Univ 28(2):139–141

Yao L, Zhou GN, Feng ZH, Gao BJ, Yuan SL (2006) Survival and development immature *Harmonia axyridis* (Pallas) feeding on *Chaitophorus popleti* (Panzen) propagated on transgenic insect-resistance hybrid poplar 741. J Agric Univ Hebei 29:73–76

Zhang HC, Liu ZS (2006) Agricultural statistics of China 2006. China Agricultural Press, Beijing, pp 230–257

Zhang Z, Wang J, Zhang J, Zhang S (2004) Effects of transgenic poplars to the structure of insect community. Sci Silvae Sinicae 40(2):84–89

Zhang BY, Su XH, Li YL, Zhang YA, Qu LJ, Wang YZ, Tian YC (2006) Production of *Populus alba* × *P.glandulosa* with a coleopterous insect resistant gene and analysis of insect resistance. J Bering For Univ 28(2): 102–105

Zheng JB, Liang HY, Gao BJ, Wang YF, Tian YC (2000) Selection and insect resistance of transgenic hybrid poplar 741 carrying two insect-resistant genes. Sci Silvae Sinicae 36(2):13–19