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Effects of insect-resistant transgenic cotton on ground-dwelling beetle assemblages (Coleoptera)

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Abstract

We compared the ground-dwelling beetle assemblages under four scenarios in which transgenic Bt (Cry 1Ac) cotton (33B), transgenic Bt (Cry 1Ac)+CpTI cotton (SGK321), conventional cotton (33), conventional cotton (Shiyuan 321) in North China. During the survey in two years (2009–2010), 24 ground beetle species were captured with pitfall traps in 20 plots which included five replicates for each cotton type. No significant difference was observed in the number of ground beetle species captured, activity density, evenness and Shannon-Wiener diversity among the four cotton varieties. *Chlaenius posticalis* was less abundant in transgenic Bt+CpTI cotton (SGK321) fields than its conventional cotton (Shiyuan 321), but more abundant in transgenic Bt cotton (33B) fields compared with its conventional cotton (33). There was no significant difference for other abundant species between in transgenic cotton and in conventional cotton fields. Based on non-metric multidimensional scaling (NMDS) analysis, ground-dwelling beetle assemblages were similar in transgenic and conventional cotton over the two years, but the ground-dwelling beetle assemblages in transgenic cotton 33B significantly differed from that in the conventional cotton (strain 33) in 2010. No strong evidence that the transgenic cotton effect on ground-dwelling beetle assemblages was found in this study.

Keywords: biodiversity, community, cotton varieties, non-target insects

1. Introduction

Transgenic cotton expressing a toxic protein derived from *Bacillus thuringiensis* (Bt) Berliner is cultivated worldwide (Lawrence 2005; Tan 2011), and approximately 70% of the cotton cultivated in China is transgenic Bt cotton (Wu 2007; Liu and Wu 2011). Transgenic Bt cotton effectively controls *Helicoverpa armigera* (Hübner), *Pectinophora gossypiella* and some Lepidopteran insects, but unlike broad-spectrum insecticides, they do little or no harm to most other insect pests (Tabashnik 2010). To increase the efficacy of transgenic crops, genes of other insecticidal proteins such as the cowpea trypsin inhibitor (CpTI) have been introduced into cotton (Ranjekar *et al.* 2003). Recently, transgenic Bt+CpTI cotton is also planted in parts of cotton-growing regions in China (Xu *et al.* 2012).

Transgenic crops not only effectively reduced the damage from target insects (Wu *et al.* 2008; Hutchison *et al.*

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2010), but also reduce the insecticide use (Huang *et al.* 2010; Tabashnik 2010). At the same time, the potential risks of transgenic plant on environment received more attention (Krebs *et al.* 1999; Mullin *et al.* 2005; Harwood *et al.* 2006). For instance, when the dominant pest, cotton bollworm (*Helicoverpa armigera*), was controlled effectively by increased area of Bt cotton in North China, the population sizes of subdominant pest, mirid bugs (Heteroptera: Miridae) in cotton and other crops have progressively increased (Lu *et al.* 2010). Another environmental concern is the potential effect of insect-resistant transgenic crops on non-target organisms (NTOs) (Snow and Morán-Palma 1997; Andow *et al.* 2006; Romeis *et al.* 2009). As Snow *et al.* (2005) suggested non-target organisms could be grouped into five categories: (a) beneficial species, including pollinators and natural enemies of pests; (b) non-target herbivores; (c) soil organisms; (d) species of conservation concern; and (e) species contributing to local biodiversity.

Ground-dwelling beetles are an important group of ground-dwelling fauna in farm ecosystems (French *et al.* 2004), and are regarded as indicator species for biodiversity in agricultural ecosystem (Roth and Perfecto 1994; Duelli and Obrist 2003; Carrière *et al.* 2009). Most of ground-dwelling beetles are carnivorous or omnivorous (Lövei and Sunderland 1996; Thayer 2005), and are beneficial to control agricultural pests (Brust *et al.* 1986; Hance 1987; Clark *et al.* 1994; Symondson *et al.* 2002); so as important no-target organisms, they can be exposed to toxins through feeding on plants or herbivorous insects, or toxin persistence in the soil (Lundgren 2005; Álvarez-Alfageme *et al.* 2009).

Several studies had assessed the impact of transgenic crops on ground-dwelling beetles, but no generalization has been reached. Several studies (Lozzia 1999; French *et al.* 2004; Mullin *et al.* 2005; Torres and Ruberson 2005b; Floate *et al.* 2007) found no serious adverse effects but in a few cases significant differences in activity abundances of ground-dwelling beetles between transgenic crops and conventional crops exist in certain years (Naranjo 2005; Torres and Ruberson 2007; Toschki *et al.* 2007). In addition, several laboratory experiments showed that toxic proteins could flow along the trophic food web from crops to ground beetles (Meissle *et al.* 2005; Harwood *et al.* 2006; Álvarez-Alfageme *et al.* 2009; Peterson *et al.* 2009).

In China, transgenic insect-resistant cotton was approved for commercial use in 1997. After its adoption, most studies ever evaluated the impacts of Bt cotton on NTO's in larger scale commercial cotton cultivation (Wu 2007; Li *et al.* 2010; Yu *et al.* 2011), but few studies on the transgenic insect-resistant cotton impacts on the ground-dwelling beetles, especially on transgenic Bt+CpTI cotton, were reported from China.

In this study, we assessed the impact of transgenic cotton on non-target ground-dwelling beetles in North China. The following questions were addressed: (1) What are the differences in the number of species captured and activity density of ground-dwelling beetle assemblages between transgenic cotton and conventional cotton? (2) Is there different impact on the ground-dwelling beetle between single-gene and stacked-gene transgenic cotton? (3) Which species dominate the transgenic cotton or conventional cotton fields? (4) Is there any negative impact from single-gene or stacked-gene transgenic cotton on the ground-dwelling beetle assemblage species composition and distribution?

2. Results

2.1. Ground-dwelling beetle fauna

In total, 1 043 individuals were captured during the two years of the study. These individuals belonged to 24 species from seven families (Table 1): Carabidae (14 spp.), Scarabaeidae (5 spp.), Geotrupidae (1 sp.), Tenebrionidae (1 sp.), Bruchidae (1 sp.), Silphidae (1 sp.) and Staphylinidae (1 sp.). In addition, other arthropods collected in pitfall traps (e.g., Formicidae, Araneae, Lepidoptera larvae, Orthortera, etc.) were also provided for comparison with other studies in Table 1.

According to the guidelines by Niemelä *et al.* (1992), species were regarded as abundant if the catch accounted for >5% in at least one cotton type, and the individuals composed more than 2% of the total catch. Thus, 11 species were defined as abundant species: *Carpelimus* sp., *Chlaenius micans* (Fabricicus), *Chlaenius posticalis* Motschulsky, *Dolichus halensis* Schaller, *Gonocephalum reticulatum* Motschulsky, *Harpalus griseus* (Panzer), *Harpalus ronicus* Bates, *Harpalus sinicus* Hope, *Pterostichus* sp., *Rhyssemus germanus* (Linnaeus) and *Scarites terricola* (Bonelli) (Table 1).

D. halensis and *C. micans* were the two most abundant species and composed more than half of the total catch. The numbers of captured specimens from both species were higher in 2010 than in 2009, and their relative frequencies were different. *D. halensis* composed 37.4% of the total catch in 2010 but only 20.1% in 2009, and *C. micans* composed 27.1% of the catch in 2009 and 24.6% in 2010 (Table 1).

Repeated-measures analysis of variance (ANOVA) showed that the catch frequency and abundance for nearly all of the abundant species were significantly affected by the year, but not by cotton type. Most species were more numerous in 2010 than in 2009, four species were the

Table 1 Count (C) and frequency (F) of the ground-dwelling beetles and other arthropods captured by pitfall trap on cotton field in Langfang, North China, in each year and each cotton types[1)]

	SY321		SGK321		33		33B		Total	
	2009	2010	2009	2010	2009	2010	2009	2010	C	F
Beetles (Coleoptera)	113	199	52	205	71	128	107	168	1043	100
Carabidae										
Chlaenius posticalis Motschulsky	3	14	2	3	1	–	4	5	32	3.1
Chlaenius micans (Fabricius)	34	58	8	37	20	26	31	51	265	25.4
Dolichus halensis Schaller	27	76	13	88	9	44	20	54	331	31.7
Harpalus corporosus (Motschulsky)	3	–	1	1	1	–	4	–	10	1.0
Harpalus griseus (Panzer)	4	2	5	4	6	2	6	1	30	2.9
Harpalus macronotus Tschitscherine	–	1	–	1	1	–	–	2	5	0.5
Harpalus nigrans A. Morawitz	1	4	–	7	1	1	–	8	22	2.1
Harpalus pallidipennis Morawitz	–	2	–	–	–	3	–	1	6	0.6
Harpalus roninus Bates	–	5	1	10	2	3	3	14	38	3.6
Harpalus sinicus Hope	4	7	1	9	1	4	2	12	40	3.8
Harpalus simplicidens Schauberger	1	–	1	1	1	1	2	4	11	1.1
Scarites terricola (Bonelli)	4	4	3	6	6	5	2	–	30	2.9
Pterostichus sp.	3	–	4	14	2	15	–	9	47	4.5
Tachys sp.	3	12	–	1	–	1	5	3	25	2.4
Tenebrionidae										
Gonocephalum reticulatum Motschulsky	10	–	8	–	4	6	4	1	33	3.2
Scarabaeidae										
Caccobius unicornis Fabricius	7	–	–	2	–	3	1	–	13	1.2
Holotrichia parallela Motschulsky	–	2	–	–	–	1	–	–	3	0.3
Onthophagus lenzii Harold	2	2	1	–	–	–	–	1	6	0.6
Onthophagus punctator Reitter	1	1	–	–	–	1	1	2	6	0.6
Onthophagus trituber Wiedeman	1	–	–	–	–	–	–	–	1	0.1
Geotrupidae										
Rhyssemus germanus (Linnaeus)	1	1	3	20	1	12	3	–	41	3.9
Silphidae										
Ptomascopus plagiatus (Menetries)	–	8	–	1	–	–	–	–	9	0.9
Staphylinidae										
Carpelimus sp.	4	–	1	–	15	–	18	–	38	3.6
Bruchidae										
Callosobrachus sp.	–	–	–	–	–	–	1	–	1	0.1
Orthoptera	167	631	113	481	130	411	123	553	2609	29.9
Dermaptera	539	772	217	446	208	453	315	519	3469	39.8
Lepidoptera larvae	23	131	4	80	3.0	61	14	137	453	5.2
Araneae	160	126	140	158	163	143	220	132	1242	14.2
Formicidae	249		141		205		348		943	10.8

[1)] SY321, conventional cotton (Shiyuan 321); SGK321, transgenic cotton (Bt+CpTI); 33, conventional cotton; 33B, transgenic cotton (Bt). The data of ants are lack in 2010. –, no specimen were collected.

reverse (Table 2). Only one species, *Chlaenius posticalis*, was significantly impacted by the cotton types ($F_{1,3}$=5.14, P=0.01). The total number of *C. posticalis* specimens collected from stacked-gene transgenic cotton SGK321 field was significantly lower than conventional cotton SY321 field (P=0.03), but specimens collected from single-gene transgenic cotton 33B field was higher than from conventional cotton 33 (P=0.02). On the other hand, specimens of *C. posticalis* collected in conventional cotton SY321 was more than in the conventional cotton 33 (P=0.002). In addition, no significant year (P=0.317) and cotton type×year interactions (P=0.390) were detected in the abundant of *C. posticalis* (Table 2).

2.2. Diversity

The repeated-measures ANOVA showed no significant differences between transgenic and conventional cotton in the number of species captured ($F_{1,3}$=0.466, P=0.710), activity density ($F_{1,3}$=1.170, P=0.352), Shannon-Wiener diversity ($F_{1,3}$=0.133, P=0.939) and evenness ($F_{1,3}$=1.339, P=0.297) over the two-year period. In addition, the collection year significantly affected ground-dwelling beetle activity density (P=0.002), but did not affect other diversity indices.

Table 2 Result from repeated-measures analysis of variance (RM ANOVA) on abundance of abundant ground beetles, 2009–2010

Species[1)]	SS[2)]	*df*	MS[3)]	*F*	*P*
Dolichus halensis					
Tests of within-subjects contrasts					
Year	1.978	1	1.978	27.581	0.000*
Year×Cotton type	0.010	3	0.003	0.421	0.741
Error	1.147	16	0.007		
Tests of between-subjects effect					
Cotton type	0.644	3	0.215	2.150	0.134
Error	1.597	16	0.010		
Chalenius micans					
Tests of within-subjects contrasts					
Year	636.561	1	636.561	34.654	0.000*
Year×Cotton type	54.963	3	18.321	0.997	0.419
Error	293.907	16	18.369		
Tests of between-subjects effect					
Cotton type	68.581	3	22.860	1.236	0.329
Error	295.855	16	18.491		
Chalenius posticalis					
Tests of within-subjects contrasts					
Year	0.006	1	0.006	1.069	0.317
Year×Cotton type	0.175	3	0.006	1.068	0.390
Error	0.874	16	0.005		
Tests of between-subjects effect					
Cotton type	0.491	3	0.164	5.139	0.011*
Error	0.509	16	0.003		
Harpalus griseus					
Tests of within-subjects contrasts					
Year	0.157	1	0.157	4.820	0.043*
Year×Cotton type	0.009	3	0.003	0.905	0.460
Error	0.521	16	0.003		
Tests of between-subjects effect					
Cotton type	0.002	3	0.0008	0.116	0.950
Error	1.045	16	0.007		
Harpalus ronicus					
Tests of within-subjects contrasts					
Year	0.554	1	0.554	18.180	0.001*
Year×Cotton type	0.199	3	0.007	2.182	0.130
Error	0.488	16	0.003		
Tests of between-subjects effect					
Cotton type	0.293	3	0.010	1.363	0.290
Error	1.146	16	0.007		
Harpalus sinicus					
Tests of within-subjects contrasts					
Year	0.417	1	0.417	5.730	0.029*
Year×Cotton type	0.007	3	0.002	0.306	0.821
Error	1.165	16	0.007		
Tests of between-subjects effect					
Cotton type	0.008	3	0.003	0.367	0.778
Error	1.227	16	0.008		
Gonocephalum reticulatum					
Tests of within-subjects contrasts					
Year	0.369	1	0.369	8.663	0.010*
Year×Cotton type	0.342	3	0.114	2.679	0.082
Error	0.681	16	0.004		
Tests of between-subjects effect					
Cotton type	0.117	3	0.004	1.021	0.409
Error	0.611	16	0.004		

(Continued on next page)

Table 2 (Continued from preceding page)

Species[1)]	SS[2)]	*df*	MS[3)]	*F*	*P*
Carpelimus sp.					
Tests of within-subjects contrasts					
Year	0.992	1	0.992	24.574	0.000*
Year×Cotton type	0.435	3	0.145	3.595	0.037*
Error	0.646	16	0.004		
Tests of between-subjects effect					
Cotton type	0.382	3	0.127	2.984	0.062
Error	0.682	16	0.004		
Rhyssemus germanus					
Tests of within-subjects contrasts					
Year	0.388	1	0.388	5.287	0.035*
Year×Cotton type	0.466	3	0.155	2.115	0.139
Error	1.175	16	0.007		
Tests of between-subjects effect					
Cotton type	0.417	3	0.139	1.845	0.180
Error	1.207	16	0.008		
Pterostichus sp.					
Tests of within-subjects contrasts					
Year	56.503	1	56.503	25.186	0.000*
Year×Cotton type	1.615	3	0.538	0.240	0.867
Error	35.895	16	2.243		
Tests of between-subjects effect					
Cotton type	2.709	3	0.903	0.460	0.714
Error	31.387	16	1.962		

[1)] Year, the study year (2009 and 2010). Cotton types include SY321, SGK321, 33 and 33B.
[2)] SS, sum of squares.
[3)] MS, mean square.
*, significantly different.

2.3. Species composition

The NMDS ordination showed that most samples of ground-dwelling beetle assemblages were mixed together during the two studied years, except the pair comparison between the single-gene transgenic cotton 33B and its conventional cotton in 2010, suggesting high similarity among the ground-dwelling beetle samples from the conventional and transgenic cotton (2009: ANOSIM R=–0.002, P=0.495; 2010: ANOSIM R=0.08, P=0.15) (Fig. 1). The species composition of ground-dwelling beetle samples from the transgenic cotton 33B was distinct from those in the conventional cotton 33 (ANOSIM R=0.31, P=0.02) in 2010 (Fig. 1-B).

3. Discussion

Our study provided a small-sized research field survey on the activity density, the number of species captured and diversity of ground-dwelling beetles exposed to the Bt or CpTI toxins. Compared with other studies on the NTOs (Naranjo *et al.* 2005; Toschki *et al.* 2007), the size of our experimental plots was smaller, but relative to some published experiments in which the smallest plot was just 0.002 ha (Wolfenbarger *et al.* 2008), our plots were above average in size. Moreover, although the small plots cannot avoid ground-dwelling beetles travelling among different plots, similar as some studies which test successfully the effects of farming practices on carabid assemblages (Raworth *et al.* 2004; Bourassa *et al.* 2008), we also believe that differences in catches between transgenic and conventional cotton reliably reflected the effects of transgenic cotton on ground-dwelling beetle.

Similar as other studies on the ground beetles in intensively cultivated fields in North China (Liu *et al.* 2010, 2012), very lower number of species and individuals were collected in our study. Our study was located in an intensively managed agricultural landscape, even if on the research farm itself, treatments were mostly rationalised, more precise and less intensive than the general farmer practice in the region. Cotton monocultures with a very high frequency of pesticide applications might result in a significant lower ground beetles diversity compared with semi-natural woodland or other intensively managed wheat/maize double-cropping fields (Liu *et al.* 2010). Moreover, the barren land of our experimental plots also may be unfavourable to ground beetles. Consequently, our results reporting no significant negative effects of transgenic cotton on the diversity of ground-dwelling beetles should be interpreted with these

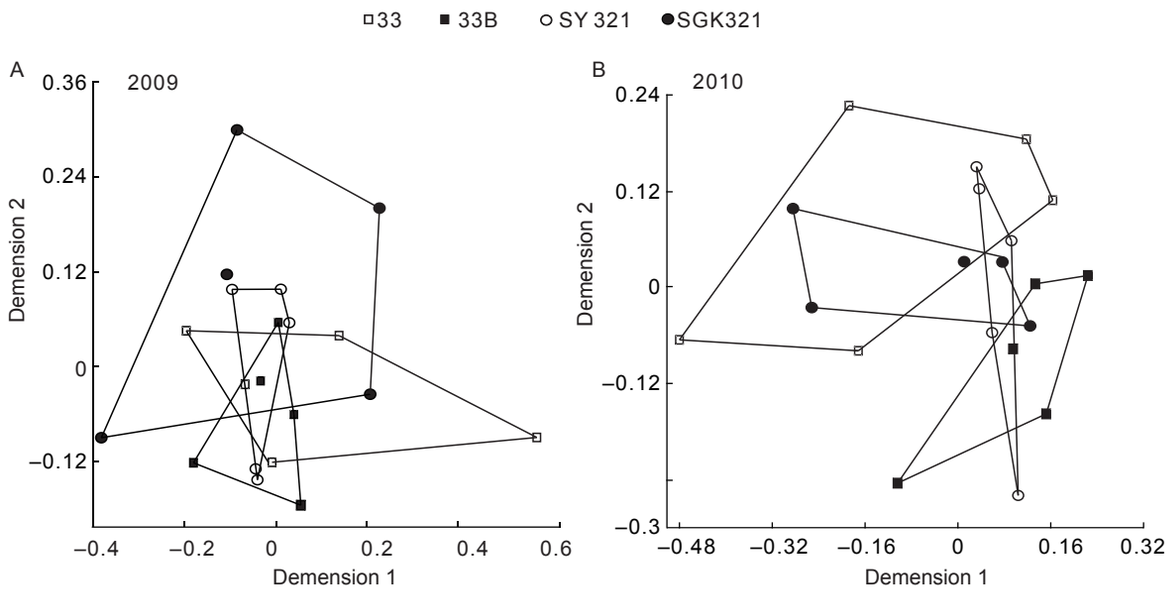

Fig. 1 Nonmetric multidimensional scaling (NMDS) for the ground-dwelling beetle among four treatments. Stress values of 2009 and 2010 are 0.2117 and 0.2209, respectively. 33, conventional cotton; 33B, transgenic cotton (Bt); SY321, conventional cotton (Shiyuan 321); SGK321, transgenic cotton (Bt+CpTI).

potentially limiting factors in mind.

No convinced and significantly negative effects of transgenic cotton on the diversity of ground-dwelling beetles were found in our study. Our results also found fluctuations in the activity density in *C. posticalis* in fields planted with single-gene and stacked-gene transgenic cotton. Positive (Torres and Ruberson 2005a; Toschki *et al.* 2007; Lu *et al.* 2012) and negative (Sun *et al.* 2003; Zhou *et al.* 2004) changes in the abundance of ground beetles and other natural enemies were reported, but in our case, the changes in the activity density of *C. posticalis* can probably be interpreted considering that this species is an important natural enemy of Lepidoptera larvae (Deng *et al.* 1983; Suenaga and Hamamura 1988). Therefore, the population increase or decrease of *C. posticalis* was possibly related with the variation of Lepidoptera larvae (Table 1). Men *et al.* (2005) also found the fourth-generation bollworms were more abundant than the second and the third generations in Bt-cotton fields. Our study also found that the abundance of Lepidoptera larvae was higher in 33B than in 33 (Table 1), similar as other studies which proved some pests have evolved resistance with widely planting of transgenic Bt crops (Bagla 2010; Storer *et al.* 2010; Tabashnik *et al.* 2010). Multi-gene stacking was used as a method to manage resistance. In fact, SGK321 was demonstrated more effective than 33B in controlling *H. armigera* (especially during the July to August) (Sui *et al.* 2008). Results showed that the abundance of Lepidoptera larvae in the SY321 field was higher than in SGK321 (Table 1), so Lepidoptera larvae change in different transgenic cotton fields may result in the different responses of *C. posticalis*. Moreover, the small plots in our study also cannot avoid *C. posticalis* travelling between different plots, and the change of microenvironment also can affect the activity density of ground beetles.

Our findings suggested a high similarity in ground-dwelling beetle communities between transgenic cotton and conventional cotton (Fig. 1), which had been observed in previous studies (AlDeeb and Wilde 2003; Candolfi *et al.* 2004; French *et al.* 2004; Torres and Ruberson 2005b, 2007; Thomazoni *et al.* 2010). However, in 2010, a significant difference was detected between transgenic Bt cotton and its conventional form (Fig. 1-B), which was similar to studies by Toschki *et al.* (2007) and Naranjo (2005) that also showed a difference for Bt plots in certain years. The variation of abundant species might explain the differences in community structure. For example, *C. posticalis* was not found in 33, but it was abundant in 33B in 2010; *Rhyssemus germanus* and *Scarites terricola* were not captured in 33B, whereas they abounded in 33 in 2010 (Table 1). The observed fluctuation in the ground-dwelling beetles may have been affected by some environmental factors, such as the litter, animal feces and land use history, which were different between the two years because the study area is ever an abandoned field and covered by sparse weeds before this study.

4. Conclusion

Overall, this study did not provide strong evidence that the transgenic cotton affected the impoverished ground-dwelling

beetle assemblages present on the site. Although the activity density of *C. posticalis* increased in transgenic Bt cotton and decreased in transgenic Bt+CpTI cotton, which might be associated with the abundance of Lepidoptera larvae or micro-environmental changes, there is no direct evidence that transgenic cotton can affect the ground-dwelling beetles. Thus, long-term monitoring in fields and laboratory experiments at the trophic level are necessary in the future.

5. Materials and methods

5.1. Field experiments

The field work was conducted at Langfang Experimental Station, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (39°30´N, 116°36´E), which is located in Hebei Province in North China.

Four different cotton varieties were cultivated on the experimental field: (1) 33B, a single-gene transgenic cotton that expresses Cry1 Ac, which had been modified from *Bacillus thuringiensis* subsp.; (2) 33; (3) SGK321, a stacked-gene transgenic cotton stacked the Bt toxin Cry1 Ac with CpTI together; and (4) Shiyuan 321 (abbreviated SY321 herein). And these two transgenic cotton varieties had been widely planted in North China (Wu 2007; Xu *et al.* 2012)

The study site comprised five replicate blocks with four plots separated by at least 2 m bare ground. The four plots in each block (interplot distances, 2.0 m) corresponded to the four varieties, 33B, SGK321, 33 and SY321. Each plot in one block was 13 m×15 m, and each plot in the other four blocks was 13 m×8 m. Thus, 20 plots per year (in 2009 and 2010) were planted during the 2-year rotation.

Four varieties of cotton were directly seeded with plastic mulching on 20 April, 2009, and 23 April, 2010, and they were maintained using standard agronomic practices; insecticide was not used during the growing season for the four cotton varieties. Plant spacing was 25 cm within each row and 50 or 70 cm between rows, which was convenient for sampling. The experimental area was abandoned field two years ago and covered by sparse weeds before our study, which may have prevented effects on the ground-dwelling beetle assemblages from agronomic practices before this study. Cotton field was managed using standard agronomic practices including field cultivator and fertilizer application.

5.2. Sampling method

Ground-dwelling beetles were collected using plastic pitfall traps (9 cm high×7.5 cm diameter) with 50% propylene glycol and plastic roofs (15 cm×15 cm). The roofs were placed 3–5 cm above the traps to protect them from litter and rain (Spence and Niemelä 1994). The cup-traps were inserted into a PVC drainage pipe longer than 9 cm to simplify the operation of the traps. The traps were emptied every two weeks, and the sampling was manipulated almost during the whole cotton growing season, i.e., from mid-July to mid-September, because ground beetles were rarely collected from cotton field after mid-September. The specimens were preserved in 70% ethanol for later identification. Although pitfall traps were biased toward active forms, and this method was not a direct measure of absolute population density, it was a useful and accurate method for monitoring and assessing local population changes in insects (Baars 1979; Spence and Niemelä 1994).

Five pitfall traps were placed in a cross shape per plot; the distance between the traps was approximately 0.5 m. Ground-dwelling beetle samples were pooled from each plot and then analyzed.

5.3. Statistical analyses

The ground-dwelling beetles collected were identified at the species level. Shapiro-Wilk was used to test normality of all variables prior to analysis. Data of species and individuals were transformed with log(x+1). Species diversity was calculated using the Shannon-Wiener diversity index (H') (Pielou 1975): $H'=-\sum P_i \ln P_i$, where P_i was the sample proportion represented by the ith species (i=1–S). Evenness was represented by $J=H'/H_{max}$ (Smith and Wilson 1996). Species richness (S) was the number of species from samples with one or more individuals (Pielou 1975). A repeated-measures analysis of variance (RM ANOVA) with the least significant difference (LSD) for post hoc pairwise comparisons was conducted on the number of species captured, activity density, Shannon-Wiener diversity index, evenness and abundance of abundant species to test the effect of time (year), cotton varieties and their interactions. An autoregressive covariance structure was used in the analysis. Time was treated as a within-subject factor; the cotton types were between-subject factors. RM ANOVA was performed using SPSS software (SPSS 1997). P<0.05 was defined as the significant level.

The ground-dwelling beetle assemblage composition was compared among transgenic and conventional cotton using a nonmetric multidimensional scaling (NMDS) plot (Clarke 1993) with the Bray-Curtis dissimilarity coefficient. Using stress levels generated by fitting the dissimilarities to distance, a two-dimensional solution was chosen as the best representation for dissimilarities among tree types. Analysis of similarity (ANOSIM) was used to test significant differences in community composition among cotton varieties. The ANMOSIM uses 10 000 random reassignments of

observed species data to assess whether rank similarities within groups (sample points of cotton type) are greater than that between groups (cotton types) (Warwick *et al.* 1990). NMDS and ANOSIM were performed using the PAST software package (Hammer *et al.* 2001).

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