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Intraspecies mixture exerted contrasting effects on nontarget arthropods of *Bacillus thuringiensis* cotton in northern China

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Abstract

1 Row-intercropping is a type of multiple cropping with two or more crops grown simultaneously in alternate rows in the same area. It is a traditional agronomic practice and is still prevalent in modern Chinese agricultural ecosystems. Many studies have proposed that intercropping at the crop species level can significantly contribute to pest management when properly managed. However, the performance of intercropping at the plant genotype level is still largely unknown.

2 A multiyear field experiment was conducted to examine the effects of intraspecies *Bacillus thuringiensis (Bt)*/non-*Bt* crop mixture on nontarget arthropods. Densities of dominant pests and predators were assessed via direct visual observations.

3 Cotton aphid population levels in monoculture *Bt* cotton fields were greater than that observed in non-*Bt* cotton, whereas the row-mixture planting of *Bt* and non-*Bt* suppressed the abundance of cotton aphids compared with that in monoculture of either genotype. Investigations also demonstrated that the intraspecies row-mixture increased whitefly abundance compared with monoculture of either genotype. However, the mixture exerted neutral effects on population sizes of mirid bugs and predators.

4 These results suggest that crop cultivation management is insufficient to control secondary pests of *Bt* cotton, and thus multiple pest suppression strategies are warranted.

Keywords *Bt* cotton, cotton aphid, intercropping, mirid bug, predator, whitefly.

Introduction

Cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), once the major cotton pest in northern China, has been effectively controlled by the adoption of transgenic cotton expressing a δ-endotoxin from *Bacillus thuringiensis* (*Bt*). However, cultivation of *Bt* cotton led to substantial variations in crop composition and pest management practices, which in turn changed the arthropod community structures within cotton ecosystems, resulting in a greater herbivore population size in *Bt* cotton compared with that in non-*Bt* cotton (Wilson *et al.*, 1992; Cui & Xia, 1998, 2000; Greene *et al.*, 1999; Herron *et al.*, 2000; Wu *et al.*, 2002; Deng *et al.*, 2003; Lu *et al.*, 2010). For example, Cui and Xia (1998, 2000) found that populations of *Aphis gossypii* Glover (Hemiptera: Aphididae), *Tetranychus cinnbarinus* Boisduval (Prostigmata: Tetranychidae), *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) and *Empoasca biguttula* Ishida (Hemiptera: Cicadellidae) were elevated in *Bt* cotton fields compared with that in non-*Bt* cotton. Herron *et al.* (2000) and Deng *et al.* (2003) found that cotton aphid populations in *Bt* cotton were significantly larger compared with non-*Bt* cotton. Wu *et al.* (2002) and Lu *et al.* (2010) reported that the widescale use of *Bt* cotton has led to a frequent outbreak of mirid bugs in northern China. However, population densities of major predator species in *Bt* cotton fields were significantly greater than those in conventional cotton receiving pesticide applications (Wu & Guo, 2005; Sisterson *et al.*, 2007;

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Lu *et al.*, 2012). Finally, evidence suggests that *H. armigera* populations in northern China have developed field-evolved resistance to *Cry1Ac*-expressing *Bt* cotton (Liu *et al.*, 2010). Therefore, the longevity of *Bt* cotton is dependent on its control effect on the resistance development of target pests and outbreaks of nontarget pests.

Various refuge strategies have been field-tested for delaying the resistance development of target pests to *Bt* crops (Gould, 1998; Tabashnik *et al.*, 2005) with promising effects (Tabashnik *et al.*, 2008, 2009; Wu *et al.*, 2008). At the same time, much effort has been directed toward managing the secondary pest complex in *Bt* cotton, and increasing the biological control effect of natural enemies is an effective strategy for overall *Bt* cotton management. Numerous studies suggest that the enhancement of predator abundance and diversity through increasing plant diversity exerts positive effects on pest control in many cropping systems (Andow, 1991; Parajulee *et al.*, 1997; Parajulee & Slosser, 1999; Men *et al.*, 2004; Gardiner *et al.*, 2009).

In most agro-ecosystems, strip intercropping, namely the planting of two or more crops simultaneously in different strips in a manner to permit independent cultivation, as well as allowing the crops to interact agronomically (Vandermeer, 1992), is the principal strategy in plant diversity enhancement. Such strip intercropping could be achieved via interspecies or intraspecies row-mixtures. An interspecies row-mixture refers to the planting of two or more species of crops simultaneously in the same field, whereas an intraspecies mixture is the planting of two or more genotypes of the same crop species simultaneously in the same field. A few studies have documented the effects of intraspecies mixtures on the predator complex and any resulting pest control in cotton fields (Sisterson *et al.*, 2004; Yang *et al.*, 2012). For cotton fields, the intraspecies row-mixture of *Bt* and non-*Bt* cotton is equivalent to setting a structured refuge.

The present study aimed to explore the feasibility of utilizing a structured refuge to suppress nontarget pests of *Bt* cotton in small-holder agro-ecosystems of northern China. We hypothesized that a row-mixture planting of *Bt* and non-*Bt* cotton would exert a positive effect on pest control, and that this effect would be irrespective of cotton genotype. We also hypothesized that the effect of an intraspecies mixture on pest and predator abundance would be consistent across growing seasons.

Materials and methods

Field experimental design

Field experiments were conducted at the Langfang Experiment Station (39.538°N, 116.708°E) of the Chinese Academy of Agricultural Sciences (CAAS), located in the Jiuzhou County of Hebei Province. Before 2008, field corn was grown in the selected fields. Based on the current *Bt* cotton adoption rate of the Hebei Province and the refuge size for target pests recommended by Vacher *et al.* (2003), we set up three experimental treatments: (i) monoculture of a *Bt* cultivar; (ii) monoculture of a non-*Bt* cultivar; and (iii) intercropping of 75% *Bt* and 25% non-*Bt*. Intercropping plots were planted in a repeated pattern: one row of non-*Bt* and then three rows of *Bt*. The pattern continued until all rows within a plot were occupied. A randomized complete block design was used with four replications. Each plot within a block encompassed approximately 0.33 ha (length 20 m, width 16.5 m), which is a typical cotton field size in the Hebei Province. Seeding was performed at a rate expected to produce 40 000 plants per planted ha. A 3-m fallow space was left between plots and among blocks to decrease insect dispersion among treatments (Wu & Guo, 2003; Li *et al.*, 2010). Cotton was maintained with agronomic practices standard to northern China, although no fungicides or insecticides were applied to the experimental plots. Plot layout and management practices were identical across all three study years.

Cotton genotypes

The cotton genotypes used in the present study included a genetically modified *Bt* cotton (cv 'GK-12', expressing a δ-endotoxin from *Bt*) and a non-*Bt* cotton (cv 'Simian-3', the parental line of 'GK-12'). The seeds of the two genotypes were provided by colleagues from the Biotechnology Research Center of CAAS. Cotton genotypes exhibited marked differences in leaf trichome density (Xue *et al.*, 2008), *Bt* toxin content (Zhang *et al.*, 2006) and associated resistance to lepidopteran species.

Arthropod sampling

Arthropods sampled included three pest species groups [cotton aphid *A. gossypii*; mirid bug complex *Lygocoris lucorum* Meyer-Dur, *Adelphocoris suturalis* Jackson and *Adelphocoris fasciaticollis* Reuter; and whitefly *Bemisia tabaci* (Gennadius) biotype B] and four predator groups [ladybirds beetles *Coccinella septempunctata* L. and *Propylaea japonica* Thunberg; lacewing *Chrysoperla sinica* (Tjeder); spiders complex and *Orius similis* Zheng]. In each growing season, arthropod sampling was conducted every 10 days from early June until mid-September, corresponding to 4 weeks after cotton seedling emergence to plant defoliation for harvest preparation. Arthropod groups were sampled by visually inspecting 20 cotton plants at five randomly chosen sampling sites distributed across the two diagonal lines of the plot (100 plants per plot) *in situ*. Because of practical concerns as a result high densities, cotton aphid and whitefly populations were quantified by visually inspecting three leaves each from the upper, middle and lower main stem portions of the plant, respectively. In total, nine leaves per selected plant were investigated. For other arthropods, entire plants were visually inspected in the morning (8.00–10.00 h) or afternoon (16.00–18.00 h), with particular attention being paid to flowers and squares, which are likely hiding places for feeding insects.

Statistical analysis

Data obtained from the 100 total plants from the five sampling sites within each experimental plot were pooled to correct for data dependency, and so each plot was used as a replication unit. Arthropod density responses to treatments were analyzed by two steps. First, the overall effects of these factors on pests and predator abundances during the 3-year study were analyzed with a linear mixed effect model using planting pattern and cotton genotype as a fixed factor, and year as a random factor (SAS Institute, 2003). Then, the effects of planting pattern (monoculture or row-mixture), cotton genotype (*Bt* or non-*Bt*), sampling date, and their interactions, on the abundance of natural enemies and herbivores in each growing season were further analyzed separately with a PROC MIXED procedure in repeated measures analysis of variance (SAS Institute, 2003). Differences in arthropod abundances on specific sampling dates were separated using Fisher's least significant difference. When necessary, the data were $\sqrt{(n+0.5)}$ transformed or $\log(n+1)$ transformed to satisfy assumptions of normality and homogeneity of variance before analysis of variance.

Results

Row-mixture intercropping arrested the abundance of cotton aphids

Cotton aphid population size varied significantly across years and sampling dates. The abundance of cotton aphid on *Bt* cotton was higher that that of non-*Bt*, and row-mixture intercropping markedly decreased the abundance of cotton aphid throughout all 3 years of the study (Figs 1 and 2). In addition, the interaction between planting pattern and cotton genotype was statistically significant (Table 1).

In each growing season, cotton aphid population levels varied significantly across sampling dates (Figs 1 and 2 and Table 2). Row-mixture intercropping significantly depressed the abundance of cotton aphid compared with the *Bt* or non-*Bt* monoculture (Fig. 1). At the same time, the densities of cotton aphid varied greatly within cotton genotypes in monoculture fields and across growing seasons (Fig. 2 and Table 2). The effect of cotton genotype on cotton aphid densities changed with sampling date, as did the effect of planting pattern. The impact of *Bt* cotton on population size of cotton aphid varied greatly among years. In 2008, the abundance of cotton aphid in *Bt* cotton was markedly higher than that in *non-Bt* cotton (Fig. 2A–D), whereas, in 2009 and 2010, no significant differences in cotton aphid were found between *Bt* and non-*Bt*. In addition, the interaction between cotton genotype and planting pattern was not significant for cotton aphid, except for the 2008 growing season (Table 2).

Row-mixture intercropping exerted a neutral effect on the abundance of mirid bugs

Abundances of mirid bugs showed significant variations across years and sampling dates, although planting pattern and genotype had no marked impact on mirid bugs activities. Yet, the interactions between year and planting pattern were statistically significant (Table 1).

Discernible fluctuations of mirid bugs abundance were found across the sampling dates for all years (Fig. 3 and Table 2), although comparable numbers of mirid bugs were found between *Bt* and non-*Bt* cotton fields at the same sampling date. Row-mixture intercropping showed no pronounced effect on the abundance of mirid bugs compared with the monocultures of

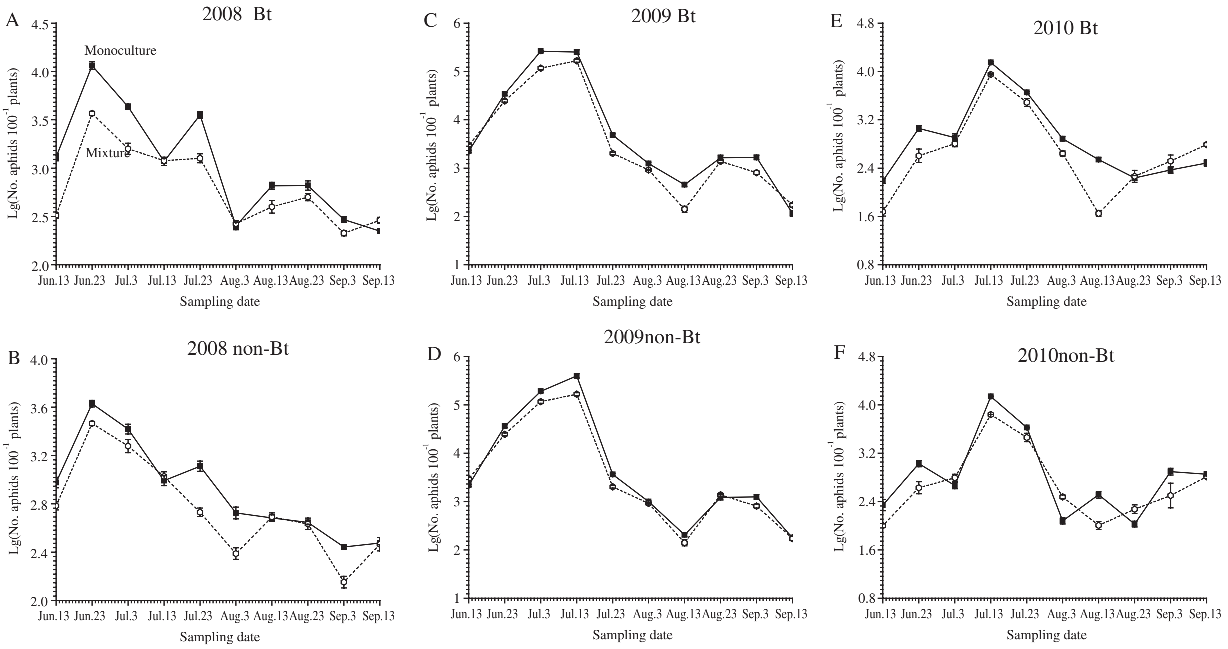

Figure 1 Dynamics of the cotton aphid on the same cotton genotype under different planting patterns [monoculture *Bacillus thuringiensis (Bt)* cultivar, monoculture non-*Bt* cultivar, and mixed-rows of same *Bt* and non-*Bt* cultivars] from mid-June to mid-September in (A, B) 2008, (C, D) 2009 and (E, F) 2010. Solid-lines on the line graphs represent population sizes (mean ± SE) of the monoculture fields, whereas the dotted-lines represent those of the mixture of *Bt* and non-*Bt* cotton at a row ratio of 75% to 25%, respectively.

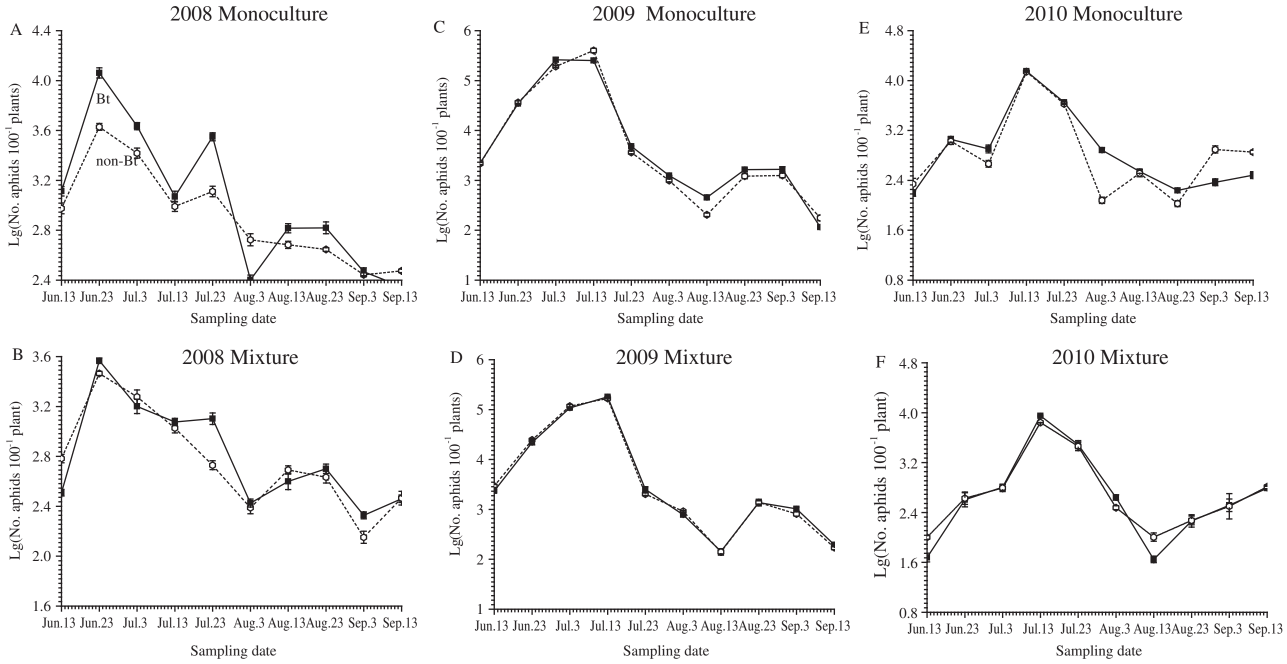

Figure 2 Dynamics of cotton aphid on *Bacillus thuringiensis (Bt)* cotton and non-*Bt* cotton under different planting patterns (monoculture *versus* mixture) from mid-June to mid-September in (A, B) 2008, (C, D) 2009 and (E, F) 2010. Solid-lines on the graphs represent population sizes (mean ± SE) of monoculture fields, whereas the dotted-lines represent those of the mixture of *Bt* and non-*Bt* cotton at a row ratio of 75% to 25%, respectively.

Table 1 *F*- and *P*-values from the linear mixed model estimated effect of cotton genotype, planting pattern, year and their interactions on population size of herbivores in northern China cotton fields in 2008, in 2009 and 2010

		Cotton aphid		Mirid bugs		Whitefly	
Factor	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Year	2,40	2300.90	< 0.0001	289.63	< 0.0001	51.28	< 0.0001
Genotype	1,40	10.66	0.002	0.08	0.781	0.49	0.488
Pattern	1,40	268.34	< 0.0001	3.85	0.057	17.64	0.001
Date	9, 459	82.77	< 0.0001	104.77	< 0.0001	618.25	< 0.0001
Year × Genotype	2,40	10.91	0.002	0.30	0.589	1.84	0.183
Year × Pattern	2,40	1.34	0.273	3.29	0.049	0.35	0.707
Genotype × Pattern	1,40	5.44	0.008	2.33	0.112	2.16	0.129
Genotype × Pattern × Year	2,40	0.18	0.835	1.57	0.221	1.01	0.372

either the *Bt* or non-*Bt* genotypes in the 2009 and 2010 growing seasons (Fig. 3 and Table 2). However, in 2008, the population size of mirid bugs was higher in intercropping fields compared with the corresponding cotton genotype in monoculture fields (Fig. 3C, D). The interaction between genotype and planting pattern was significant for the growing season of 2008 (Table 2). In conclusion, no clear trends were found because the effect of cotton genotype and planting pattern on the population size of mirid bugs changed with sampling date.

Row-mixture intercropping increased abundances of whiteflies

The results of a linear mixed model indicated that there were significant variations in the abundances of whiteflies across years and sampling dates. In addition, the row-mix planting pattern consistently showed increased whitefly densities. However, differences as a result of cotton genotype were not detectable. Furthermore, neither the interactions between each of two factors (year, planting pattern and genotype), nor the interactions of all the factors were statistically significant (Table 1).

Whitefly densities varied significantly across sampling dates. There were significant differences in abundance of whitefly between the two planting patterns (monoculture versus mixed-row plantings) in most of the investigating periods (Fig. 4 and Table 2). The row-mixture plantings increased the abundance of whitefly compared with the same genotype monocultures, whereas the effect of cotton genotype on whitefly abundance was negligible in most cases, whether under monoculture or mixture. Moreover, the interaction between planting pattern and

Table 2 *F*-values of the repeated measures analysis of variance testing the effects of planting pattern, cotton genotype and sampling date on population sizes of cotton aphid, mirid bugs and whitefly in northern China cotton fields in 2008, 2009 and 2010

Year	Factor	d.f.	Cotton aphid	Mirid bugs	Whitefly
2008	G	1,12	39.46***	4.7	5.93*
	P	1,12	238.67***	5.21*	16.45**
	D	9,108	501.33***	149.26***	2875.59***
	G × D	9,108	18.41***	4.14***	7.14***
	P × D	9,108	19.01***	14.34***	2.88**
	G × P	1,12	11.34**	7.65*	0.39
	G × P × D	9,108	9.98***	8.62***	2.36*
2009	G	1,12	3.59	1.11	0.85
	P	1,12	88.03***	3.55	1.52
	D	9,108	3829.37***	128.47***	389.24***
	G × D	9,108	6.09***	2.78**	0.32
	P × D	9,108	19.44***	4.90***	7.12***
	G × P	1,12	2.75	0.02	0
	G × P × D	9,108	6.26***	4.24***	0.92
2010	G	1,12	0.04	0.98	0.88
	P	1,12	42.55***	1.46	18.86***
	D	9,108	404.33***	218.71***	668.19***
	G × D	9,108	13.10***	3.01**	0.5
	P × D	9,108	20.35***	14.81***	11.72***
	G × P	1,12	1.79	0.75	2.59
	G × P × D	9,108	7.74***	2.59**	1.34

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

G, cotton genotype; P, planting pattern; D, sampling date; G × D, interaction between cotton genotype and sampling date; P × D, interaction between planting pattern and date; G × P, interaction between cotton genotype and planting pattern. G × P × D, interaction between cotton genotype, planting pattern and sampling date.

sampling date was significant for all growing seasons. However, the interactions between planting pattern and sampling date, and between cotton genotype, planting pattern and sampling date, were only significant for the 2008 growing season.

Row-mixture intercropping failed to enhance the abundance of predators

Overall, the predator abundance significantly varied between treatments among years and across sampling dates (Table 3). Cotton genotype and planting pattern contributed little to the variances in predator abundance, and this effect was consistent among growing seasons. However, the interactions between year and planting pattern were significant for most of the taxa group (Table 3).

The densities of all predator taxa fluctuated greatly across sampling dates (Table 4) but did so equally between monoculture and row-mixture intercropping fields, whether for *Bt* or non-*Bt* cotton fields in most cases, except for the growing season of 2008 (Table 4). The abundance of predators, such as adult ladybirds, *O. similis* Zheng and spiders, was higher in the non-*Bt* cotton field compared with that in the *Bt* field, whether for monoculture or mixture fields in 2008. The effect of planting pattern on the population size of adult ladybirds changed with sampling date for the 2008 and for 2010 growing seasons. At the same time, the effect of cotton genotype and planting pattern on spiders abundance changed with sampling date, and no clear trends were found for all the years tested.

Discussion

Impact of row-mixture as a Bt resistance management approach on cotton aphid

Cotton aphid abundance was higher on *Bt* cotton than on non-*Bt* cotton in 2008, whereas, in 2009 and 2010, the population size of cotton aphid in *Bt* fields was similar to that of the non-*Bt* fields. Many other studies have also reported that the abundance of cotton aphid in *Bt* cotton is higher compared with that in conventional non-*Bt* cotton (Wilson *et al.*, 1992; Cui & Xia, 1998; Greene *et al.*, 1999; Deng *et al.*, 2003). The discrepancy observed among the seasons in the present study may be a result of varying environmental conditions and arthropod complexes across study years.

Furthermore, intraspecies intercropping has suppressed the abundance of cotton aphid. This result supports our hypothesis that intraspecies mixtures would improve pest suppression. This finding is congruent with previous studies indicating that intercropping exerts strong positive effects on pest control (Litsinger & Moody, 1976; Risch, 1981; Andow, 1991; Altieri & Nicholls, 2004; Bomford, 2004; Shrewsbury & Raupp, 2006; Björkman *et al.*, 2010). However, the effects of mixed-row intercropping showed a significant variation among years and within genotypes. Xue *et al.* (2008) stated that the outbreak of cotton aphid was more frequently observed in transgenic *Bt* cotton because the lower leaf trichome density of transgenic *Bt* cotton facilitated aphid feeding compared with conventional non-*Bt* cultivars. Accordingly, we would have expected an

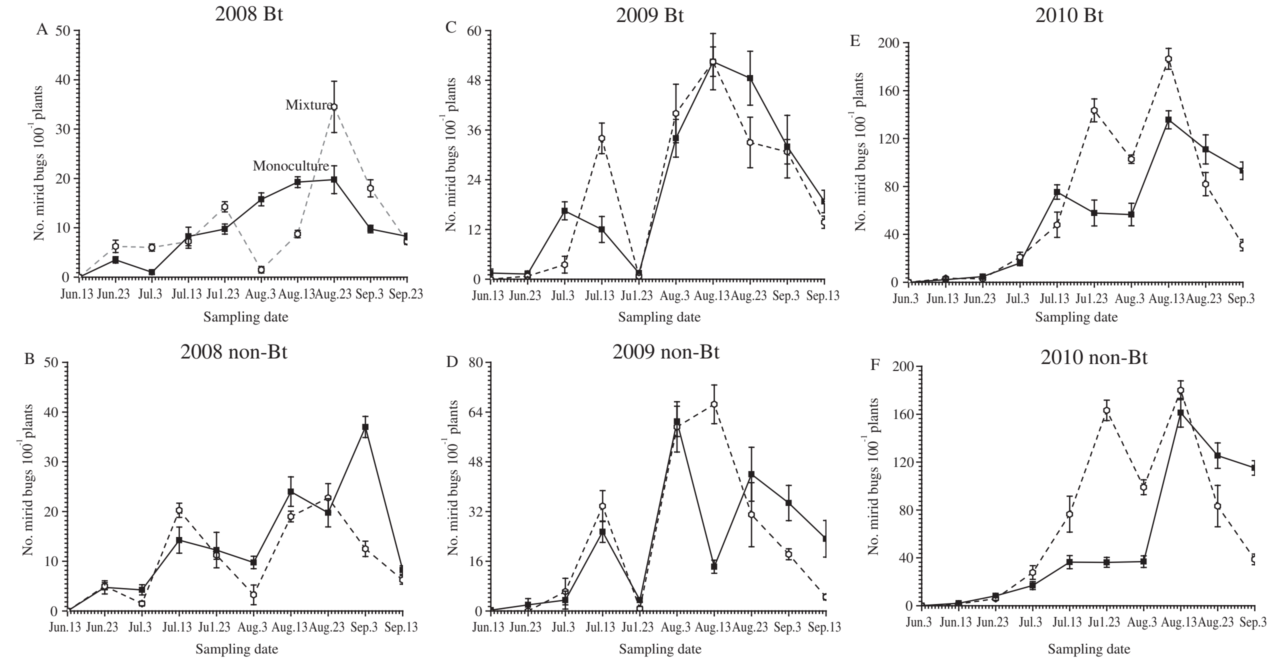

Figure 3 Dynamics of mirid bugs on *Bacillus thuringiensis (Bt)* cotton and non-*Bt* cotton with different planting patterns (monoculture *Bt* cultivar, monoculture non-*Bt* cultivar, and mixed-rows of same *Bt* and non-*Bt* cultivars) from mid-June to mid-September in (A, B) 2008, (C, D) 2009 and (E, F) 2010. Solid-lines on the line graphs represent population sizes (mean ± SE) of the monoculture fields, whereas the dotted-lines represent those of the mixture fields of *Bt* and non-*Bt* cotton at a row ratio of 75% to 25%, respectively.

intermediate cotton aphid population in mixture plots, with the highest densities in *Bt* plots and the lowest densities in non-*Bt* plots. However, the suppression effect of mixture on cotton aphid was similar between the two genotypes. This indicates that there may be other factors contributing to the observed phenomenon.

Influence of row-mix intercropping on whiteflies and mirid bugs

By contrast to our hypothesis, intercropping increased the occurrences of whitefly in the present study. At the same time, intercropping failed to alter the abundances of mirid bugs. The specific response of pests to intercropping may result from dispersion capability differences. Furthermore, the effect of intercropping on pests is partly determined by plant resistance, whereas plant resistance changes with the developmental age of plant (Barton & Koricheva, 2010). In addition, plants can modulate their defensive strategy based on neighbour identity (Broz *et al.*, 2010). The discrepancy of mixed-row plantings on mirid bugs among seasons may be the result of variation in climate and interactions among arthropods.

Neutral effects of row-mix intercropping on predator abundance

Planting pattern did not significantly influence the predator abundance in most of cases. Therefore, our expectation of increased predator activities in intercropped fields was rejected. Takizawa and Snyder (2011) suggested that higher predator biodiversity fostered the survivorship of juveniles, which in turn increased reproductive rates and contributed more offspring to succeeding generations, along with an increased foraging efficiency. In the present study, the abundances of predators, such as ladybirds and spiders, in intraspecies mixture cotton fields were higher than the corresponding genotype of monoculture cotton fields in 2008. However, this phenomenon was not observed in 2009 and 2010. In general, lower prey abundances are expected to aggravate intraguild predation and competition and thus lead to reduced activity and lower reproduction rates. Considering all of the factors noted previously, it is not unexpected that the intraspecies plantings in the present study did not enhance the occurrence of predators when prey is not sufficient.

Implications for future pest management

Although the widespread planting of *Bt* cotton has led to area-wide population suppression of key target pest species, such as *H. armigera* (Wu *et al.*, 2008), *Bt* cotton adoption has also led to the outbreak of mirid bugs (Wu *et al.*, 2002; Lu *et al.*, 2010) . Therefore, management of nontarget pests is a new requirement for the sustainable application of *Bt*-transgenic cotton. From the perspective of delaying resistance development in a target pest, Wu *et al.* (2008) argued that no structured refuge is advisable as a result of the presence of natural refuges provided by the wide diversity of crops in northern China. However, other studies report that the widescale planting of *Bt* cotton has led to an increased resistance frequency in target pests in some regions

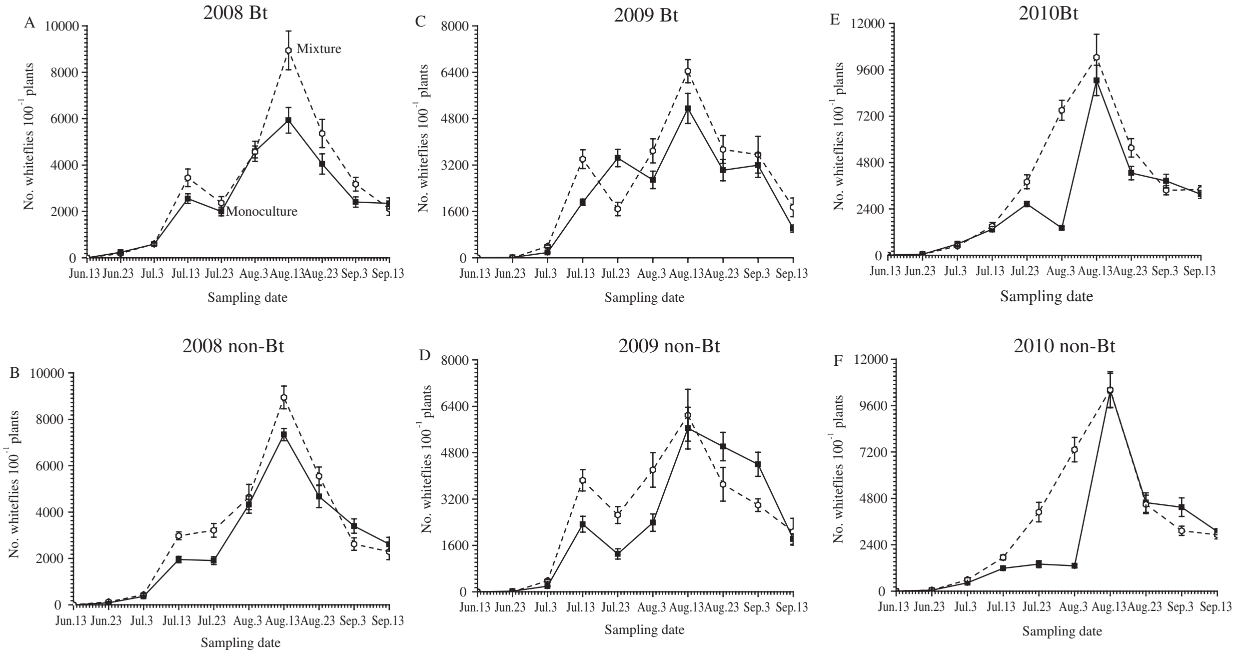

Figure 4 Dynamics of whitefly on *Bacillus thuringiensis (Bt)* cotton and non-*Bt* cotton with different planting patterns (monoculture *Bt* cultivar, monoculture non-*Bt* cultivar, and mixed-rows of same *Bt* and non-*Bt* cultivars) from mid-June to mid-September in (A, B) 2008, (C, D) 2009 and (E, F) 2010. Solid-lines on the line graphs represent population sizes (mean ± SE) of the monoculture fields, whereas the dotted-lines represent those of the mixture fields of *Bt* and non-*Bt* cotton at at a row ratio of 75% to 25%, respectively.

Table 3 *F*-values from the linear mixed model estimated effect of cotton genotype, planting pattern, year and their interactions on population size of predators in northern China cotton fields in 2008, 2009 and 2010

Factor	d.f.	Adult ladybirds	Larval ladybirds	Adult lacewing	Larval lacewing	*Orius similis*	Spiders
Y	2,40	410.97***	5.35**	60.04***	56.6***	264.99***	686.19***
G	1,40	0.85	1.50	0.87	6.91**	0.07	2.00
P	1,40	0.07	0.31	3.4	0.72	0.87	0.71
D	9,459	6.96***	9.32***	11.03***	5.01***	49.88***	101.28***
Y × G	2,40	0.00	0.01	0.15	11.93***	2.62	1.16
Y × P	2,40	7.89***	3.49*	0.59	4.78**	9.27***	43.04
G × P	1,40	0.69	0.02	0.09	8.04***	0.83	3.48*
G × P × Y	2,40	1.39	0.25	0.54	3.54*	3.08	7.17**

*P < 0.05; **P < 0.01; ***P < 0.001.

G, genotype; P, planting pattern; D, sampling date; G × D, interaction between cotton genotype and sampling date; P × D, interaction between planting pattern and sampling date; G × P, interaction between cotton genotype and planting pattern. G × P × D, interaction between cotton genotype, planting pattern and sampling date.

(Liu *et al.*, 2010; Wan *et al.*, 2012). To suppress secondary pest and to delay the resistance development of target pests, Wang *et al.* (2006) proposed that non-*Bt* crops should be planted concurrently with *Bt* crops.

The present study simulated the effects of structured refuge on secondary insects and their predators through intraspecies intercropping in northern China. The mixture in the present study's field scale (small scale) significantly suppressed the abundance of cotton aphid during the seedling and squaring (budding) stages and triggered higher whitefly densities, although it did not modify the population size of mirid bugs and the predator complex. These study results partly support the conclusion that the intraspecies mixture has a potential positive effect on pest control, although the effects are inconsistent with pest species and plant developmental stages. Therefore, future studies need to examine approaches that synchronize pest management regimes, pest species and plant developmental stages with respect to developing effective pest control programmes. In addition, a larger scale study may better determine the observed phenomenon to mimic the actual production scale. This is because the size and scope of intercropping can change the composition and diversity of landscape vegetation parameters. Because landscape structure dramatically influences the abundance, diversity and function

Table 4 *F*-values of the repeated measures analysis of variance testing the effects of cotton genotype, planting pattern, sampling date and their interactions on population sizes of predator in northern China cotton fields during in 2008, 2009 and 2010

Year	Factor	d.f.	Adult ladybirds	Larval ladybirds	Adult lacewing	Larval lacewing	*Orius similis*	Spiders
2008	G	1,12	8.35*	4.09	0.67	2.97	13.01**	5.69*
	P	1,12	11.45**	9.83**	3.2	2.07	1.8	60.69***
	D	9,108	14.42***	49.11***	14.51***	10.78***	25.25***	364.99***
	G × D	9,108	2.25*	10.38***	3.86***	2.09	5.74***	3.89***
	P × D	9,108	1.94	13.99***	0.43	3.41***	1.71	7.38***
	G × P	1,12	1.09	1.25	1.17	0.03	0.04	26.81***
	G × P × D	9,108	2.98**	0.9	1.65	2.48*	1.82	3.45***
2009	G	1,12	0.04	0.73	0.45	7.79*	1.94	4.46
	P	1,12	0.07	3.4	1.02	2.2	0.05	8.25*
	D	9,108	15.09**	18.58***	5.37***	12.53***	130.64***	408.59***
	G × D	9,108	1.23	1.03	1.01	1.77	8.68***	8.89***
	P × D	9,108	1.78	1.09	0.31	3.39***	23.18***	13.55***
	G × P	1,12	0.9	0.14	5.35*	4.80*	5.22	0.38
	G × P × D	9,108	0.71	0.6	1.3	1.91	3.50***	6.91***
2010	G	1,12	0.03	0.62	0.76	0.63	0.63	0.02
	P	1,12	2.63	2.78	3.93	1.19	2.51	0.37
	D	9,108	41.17***	16.4***	12.33***	8.49***	104.73***	0.85
	G × D	9,108	1.01	0.48	0.64	0.5	1.25	1.95*
	P × D	9,108	8.3***	1.17	1.77	1.99*	8.83***	1.67
	G × P	1,12	0.23	0.09	0	2.25	0.65	0.22
	G × P × D	9,108	0.95	0.21	0.64	0.39	1.41	2.87**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

G, genotype; P, planting pattern; D, date; G × D, interaction between cotton genotype and sampling date; P × D, interaction between planting pattern and sampling date; G × P, interaction between cotton genotype and planting pattern. G × P × D, interaction between cotton genotype, planting pattern and sampling date.

of natural enemies within croplands, optimizing the landscape structure through a reasonable arrangement of crop species or variety is crucial for developing ecologically intensive pest management approaches. Therefore, broadening the species pool of beneficial insects supported by a complex landscape and optimizing their activity should help to realize the benefits of habitat management.

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