



The impact of Bt maize expressing the Cry1Ac protein on non-target arthropods

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Abstract

From 2014 to 2016, individuals of the principal non-target arthropod (NTA) species in a field of Bt maize expressing the Cry1Ac protein (Bt38) were compared to those in a control field of the corresponding non-transformed near isoline (Z58). For all 3 years, the population dynamics and biodiversity of NTAs were analyzed to determine if any differences might be attributable to the genetically modified (GM) maize being evaluated. The main NTAs in these fields were Aphidoidea, Pentatomidae, Araneae, and Coccinellidae. Temporal variation in NTA diversity across sample dates within a year showed no significant differences between the Bt maize and the non-Bt maize field in the total number of individuals of the dominant arthropod species per 25 plants, the Shannon index (*H*), Pielou index (*J*), Simpson index (*D*), and Bray-Curtis index. The cultivation of Bt corn failed to show any detrimental evidence on individuals, *H*, *J*, *D*, and Bray-Curtis index of NTAs, and these parameters were identical in Bt and non-Bt corn plots. These results provide further evidence of the lack of ecological impact of GM maize.

Keywords Bt38 · Non-target arthropods · Arthropod diversity · Abundance

Introduction

Global area of biotech/GM crops continued to rise in 2017, reaching 189.8 million hectares compared to 185.1 million hectares in 2016, and sixty point six million hectares of GM corn was contained. In 2017, farmers in the USA planted the greatest area of GM crops, while Chinese farmers planted the eighth largest area, 2.8 million hectares (James 2018). The first GM corn was the YieldGard, which was developed by the US Food and Drug Administration (FDA) in 1996.

China's Ministry of Agriculture granted two biosafety certificates approving Bt rice and phytase maize in November 2009.

Although GM crops have been commercially planted at a large scale in recent decades, their safety is still controversial in certain countries and within certain social groups (Lucht 2015). For this reason, China has conducted safety evaluation studies on GM crops since the late 1990s. Currently, there are three groups of non-target arthropods (NTAs) whose response to GM crops is being assessed. The first group is comprised of non-target phytophagous insects (e.g., aphids, leafhoppers, capsid bugs). The second group is comprised of non-target natural enemy insects (e.g., flower bugs, lacewings, ladybird beetles, parasitoids). Assessment of the impact of GM crops on natural enemies is based on their importance to biological control. The third group consists of other economic or socially valued non-target insects such as bees, silkworms, and butterflies (Wolfenbarger et al. 2008; Arpaia et al. 2018). These groups of NTAs encompass the ecological functions of herbivores, predators, and parasitoids in maize agro-ecosystems, and may serve as representative taxa of maize agro-ecosystems, supporting that analysis of relevant data generated in one region can be transportable for the risk assessment of the same or similar GM crop products in another region (Ahmad et al. 2016).

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A growing number of studies have examined the non-target impact of Bt maize in the field (Leclerc et al. 2017; Arias-Martín et al. 2018). For example, Leclerc et al. (2017) highlighted that the optimal spatial distribution of GM fields that mitigates the risk depends on our knowledge of the habitats of NTAs and finally moderate the influence of the dispersal kernel function. Moreover Arias-Martín et al. (2018) reported a farm-scale evaluation of the impact of Cry1Ab Bt maize on canopy NTAs through a 3-year study.

A new corn variety producing Cry1Ac toxins (Bt38), which was mainly targeting *Ostrinia furnacalis* (Guenée) and *Helicoverpa armigera* (Hübner), has been developed in recent years (Chen et al. 2016). Although commercialization of Bt maize has not been allowed in China yet, field experiments have been approved to monitor its impact on target pests and non-target organisms. In the near future, they may be commercially available in China. Before commercialization, field studies are necessary to monitor the environmental risks of the Bt maize. Therefore, we conducted the study to assess the risks of this new corn variety on NTAs. The impact of Bt maize expressing the Cry1Ac protein on NTAs should be examined before this variety becomes widely adopted. We therefore intend to conduct more realistic, ecologically meaningful, and long-term detailed experiments in this area. The Bray-Curtis index is a measure of dissimilarity between two samples, making it more suitable to investigate the impact of Bt corn on NTAs and their dissimilarities over time (Yoshioka 2008). Here, the non-target arthropod communities, abundance of selected non-target arthropod groups, and Bray-Curtis dissimilarity in arthropod diversity in Bt38 and Z58 maize field were investigated and analyzed.

Materials and methods

Maize variety

Bt maize expressing the Cry1Ac protein (Bt38) and the recipient variety (Z58) were both provided by the China National Maize Center in Haidian District, Beijing. Using the evaluation criteria of maize whorl-stage resistance to Asian maize borer (*O. furnacalis*) by He et al. (2003), we found that the Bt maize variety Bt38 is resistant to this pest, while the original (pre-transformation) variety Z58 showed susceptibility (Chen et al. 2016).

Experimental location and crop cultivation methods

Experiments were conducted at Shangzhuang Experimental Station of China Agricultural University, located east of Xinlitun village, Haidian District, Beijing (altitude, 47 m; 116° 17' 52.84" E; 39° 57' 52.84" N). The study location

was in northern China and has a temperate monsoon climate and alkaline soil.

Summer maize was planted annually from 2014 to 2016. The planting dates were 15 June 2014, 15 June 2015, and 12 June 2016. In 2014 and 2015, Bt38 and Z58 were each planted in four plots, while in 2016, Bt38 and Z58 were each planted in three plots. The experiment was carried out in a randomized complete block design. Each plot was 10 × 15 m, with a between-row spacing of 60 cm and a within-row spacing of 25 cm. Three-meter bare borders were established to serve as isolation among plots. A 3-m strip border around the perimeter was planted with non-Bt maize. No crops were planted in the field until the experiment was started. No herbicides or pesticides were applied during the period of maize growth. Before planting, rotary tillage was used to control weeds.

Sampling design

Arthropod numbers were investigated and recorded by direct visual examination, with some modifications. In each plot, 125 maize plants were sampled in an “X” pattern that covered the whole plot. To avoid edge effects, the sampling started at approximately 2 m into the plot. The number and species of all the chosen non-target arthropods above ground on each plant were determined by careful searching of the maize stems and both sides of the leaves. Active insects were identified first. Unknown species were collected and preserved in 75% alcohol for later identification in the laboratory. The sampling took place once a week from 10 days after the five-leaf stage (about 40 days after cultivation) through crop maturity, which corresponded with the early vegetative, middle vegetative, late vegetative, reproductive tasseling, reproductive silk, and maturity crop stages. The number of sampling dates differed among years, ten, five, and seven times respectively. In 2015 and 2016, there was more precipitation, leading to fewer samples. Sampling dates between 2014, 2015, and 2016 are shown in Table S1.

Statistical analyses

Three indices were used to analyze the diversity of the three insect groups (herbivores, natural enemies, and a miscellaneous grouping of other economically or socially important insects): (1) the Shannon diversity index (H) (Shannon and Weaver 1949), H is the most sensitive to changes in the importance of the rare species in the sample; (2) the Pielou evenness index (J) (Pielou 1966), J describes the relative abundance or ratio of individuals in a species, which is often used in combination with H ; and (3) the Simpson diversity index (D) (Simpson 1949; Simpson and Cracraft 1995), D is the most sensitive to changes in the most abundant species in the sample, while being less sensitive to species richness.

H of the arthropods was calculated using Eq. (1)

$$H = -\sum_{i=1}^n P_i \ln P_i \quad (1)$$

J of the arthropods was calculated as per Eq. (2)

$$J = H / \ln S \quad (2)$$

D of the arthropods was calculated as per Eq. (3)

$$D = 1 - \sum_{i=1}^n \frac{N_i(N_i-1)}{N(N-1)} \quad (3)$$

H represents the Shannon diversity index, P_i is often the proportion of individuals belonging to the i th species of the total number of individuals. J represents the Pielou evenness index, and S is the total number of species in a community. D represents the Simpson diversity index, N_i is the number belonging to the i th type and N is the total number in the region. H , D , and J were calculated using diversity function of the Vegan package in R (linear mixed models: lmer function of R package *lme4*) (Oksanen et al. 2018). On each sampling date, mean values of H , J , and D were compared using one-way ANOVA to detect significant differences between Bt38 and non-Bt maize plots. SPSS 21.0 (SPSS Inc., IL, USA) was used for the calculations and analysis.

The Bray-Curtis index is an important index used to compare the differences between two samples in cluster analysis, ranging between 0 (similar) and 1 (different) (Bray and Curtis 1957). The change in the difference of the arthropod community in Bt38 and Z58 corn field was calculated using the Bray-Curtis index. The first operation was performed between Bt38 and Z58 maize fields to calculate the parameter distance between them. For each sampling date, the average and standard error of the Bray-Curtis distance was calculated. A second operation was also performed for each maize variety (Collins et al. 2000). For each sampling date, the Bray-Curtis distance of different repetitions was calculated, and then using the calculation of slope, the correlation coefficient to fitting numerical linear regression.

In this study, the average and P values of each community index of non-target arthropods were analyzed by SPSS 21.0. All other operations were analyzed with R 3.5.1 (R Core Team 2017).

Results

The non-target arthropod communities in Bt38 and Z58 maize

In the 3 years of this study, 11 families from four orders were identified and recorded, based on our choice of non-target species we wished to sample. The most abundant groups were Aphidoidea, Pentatomidae, Araneae, and Coccinellidae. Aphidoidea, Pentatomidae, and Araneae abundance in Bt38 showed no significant difference from that in Z58 for all 3 years. Coccinellidae abundance in Bt38 was not significantly different from that in Z58 for 2014 and 2015, but the individual numbers in 2016 were significantly higher in Z58 than those in Bt38 ($F = 7.49$, $df = 21$, $P = 0.02$) (Table 1).

Abundance of selected non-target arthropod groups in maize

In 2014, H of Bt38 plots was in general not significantly different from Z58 plots, but on September 2, it was significantly higher in Bt38 than that in Z58 plots (Fig. 1) ($F = 26.16$, $df = 21$, $P = 0.002$). In 2015, data were more variable but there were no significant differences between Bt38 and Z58 plots. In 2016, H of Bt38 was not significantly different from that of Z58.

In 2014, on sample dates July 8 ($F = 10.61$, $df = 21$, $P = 0.023$) and August 19 ($F = 8.45$, $df = 21$, $P = 0.027$), J of Z58 was significantly higher than that of Bt38 while on September 2, it was significantly lower than that in the Bt38 plots ($F = 25.5$, $df = 21$, $P = 0.002$) (Fig. 2). There were no significant differences between the two treatments in 2015 and 2016.

Table 1 Total arthropod individuals of dominant groups per 25 plants in Bt38 and Z58

	Maize variety	Taxon			
		Aphidoidea	Pentatomidae	Araneae	Coccinellidae
2014	Z58	140.43 ± 191.66	7.25 ± 9.40	6.93 ± 9.40	3.05 ± 5.11
	Bt38	151.5 ± 200.46	5.02 ± 6.02	4.98 ± 7.08	6.35 ± 11.12
2015	Z58	20.58 ± 14.10	6.35 ± 12.6	6.1 ± 4.69	5.1 ± 6.44
	Bt38	17.67 ± 12.20	4.25 ± 9.69	5.95 ± 4.58	4.5 ± 5.79
2016	Z58	95.62 ± 133.53	1.2 ± 2.11	10.24 ± 9.23	24.62 ± 21.09*
	Bt38	59.38 ± 124.38	1.10 ± 1.73	9.62 ± 9.6	6.1 ± 6.45

Significant differences base on repeated measures ANOVA using LSD analyses between Bt38 and Z58; * $P < 0.05$

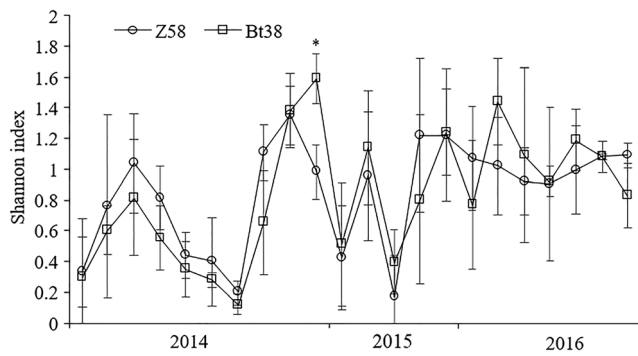


Fig. 1 The Shannon index of arthropods in Bt38 and Z58 maize in China. Changes in mean ± SE ($n=3-4$) values of descriptors of the NTA communities in Bt38 and Z58 maize. Statistically significant differences according to one-way ANOVA: $*0.01 < P \leq 0.05$

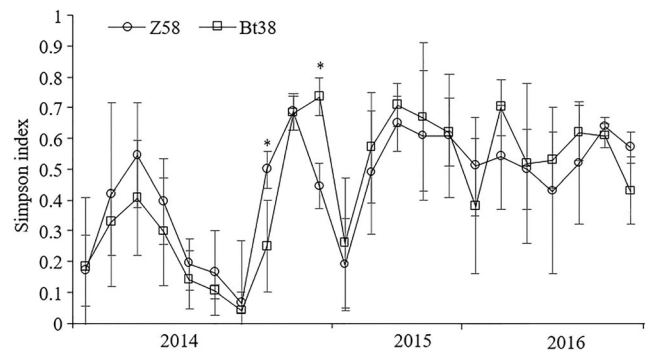


Fig. 3 The Simpson index of arthropods in Bt38 and Z58 maize in China. Changes in mean ± SE ($n=3-4$) values of descriptors of the NTA communities in Bt38 and Z58 maize. Statistically significant differences according to one-way ANOVA: $*0.01 < P \leq 0.05$

In 2014, D was significantly higher in Z58 plots than that in Bt38 plots on August 19 ($F=9.57$, $df=21$, $P=0.021$) (Fig. 3). On September 2, 2014 ($F=36.53$, $df=21$, $P=0.001$), D was significantly lower in Z58 plots than that in Bt38 plots. There were no significant differences in D between the two treatments in 2015 and 2016.

The species richness of Bt38 and Z58 was not significantly different in all 3 years (Fig. 4).

In all 3 years, maize variety had no significant effect on any of the descriptors, except species richness (Table 2). In contrast, time had a highly significant effect on all the descriptors while the interaction of time and variety was not significant for the descriptors on the whole.

Bray-Curtis dissimilarity in arthropod diversity between Bt38 and Z58 maize

The Bray-Curtis dissimilarity of Bt38 and Z58 maize changed with time (Fig. 5), but there was no significant difference between the two maize treatments throughout the study period (Spearman $r=0.28$, $P=0.068$). The change between Bray-Curtis dissimilarity and time lag among sampling dates was highly significant for both Bt ($P < 0.05$) and non-Bt ($P < 0.05$)

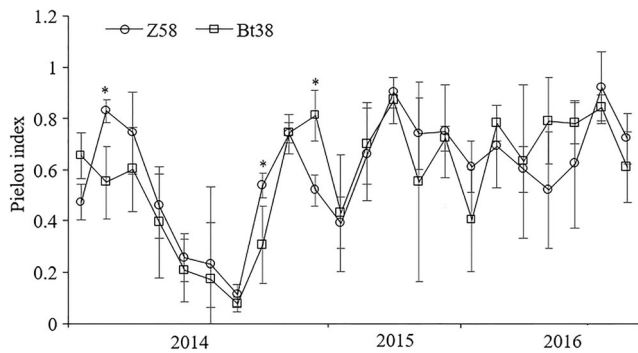


Fig. 2 The Pielou index of arthropods in Bt38 and Z58 maize in China. Changes in mean ± SE ($n=3-4$) values of descriptors of the NTA communities in Bt38 and Z58 maize. Statistically significant differences according to one-way ANOVA: $*0.01 < P \leq 0.05$

corn plots (Fig. 6), showing that the structure of the NTA communities evolved with time in the two corn plots (Fig. 6).

Discussion

Lady beetles, pirate bugs, and spiders are the most frequent predators on maize during all phenological stages of the crop (Guo et al. 2016). We found that bug and spider abundance in Bt38 showed no significant difference from that in Bt maize for all 3 years, but the individual numbers of lady birds in 2016 were significantly higher in non-Bt maize than those in Bt maize (Table 1). On some sample date, the differences of diversity indices between Bt38 and Z58 were significant (Figs. 1, 2, 3, and 4). The difference of the number of dominant species or diversity indexes of NTAs between Bt corn and non-Bt maize plots was also found in the field in past studies, In Germany, a 3-year study of above-ground arthropods in fields of Bt maize and non-Bt maize showed that in the first year, the species richness of spiders and carabid beetles in Bt maize was significantly different from that in non-Bt maize, but in the second and third years, this was not the case (Toschki et al. 2007). Guo et al. (2014) also reported some

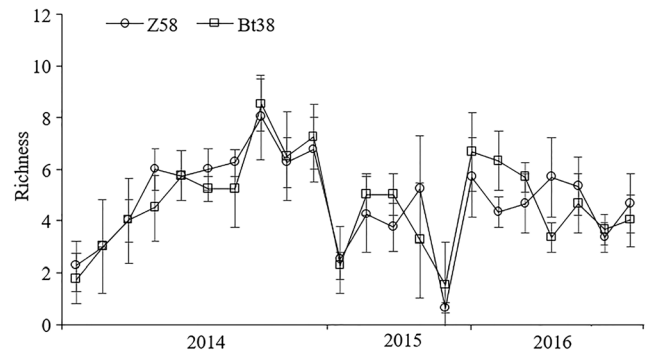


Fig. 4 The species richness of arthropods in Bt38 and Z58 maize in China. Changes in mean ± SE ($n=3-4$) values of descriptors of the NTA communities in Bt38 and Z58 maize. Statistically significant differences according to one-way ANOVA: $*0.01 < P \leq 0.05$

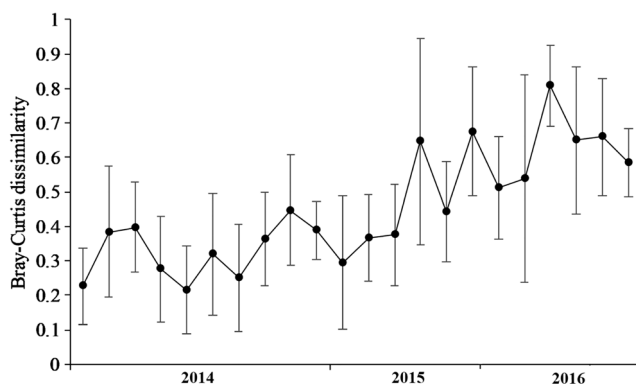
Table 2 Mean \pm SE of non-target arthropod community descriptors in Bt38 and Z58 maize in China

NTA community descriptors	Z58	Bt38	Time effect			Variety effect			Time \times variety		
			<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
Species richness (<i>S</i>)	4.79 \pm 1.65	4.72 \pm 1.75	11.95	21	<0.05	0.07	1	0.8	1.39	21	0.14
Shannon's diversity index (<i>H</i>)	0.84 \pm 0.35	0.82 \pm 0.41	10.86	21	<0.05	0.12	1	0.73	1.42	21	0.12
Pielou's evenness index (<i>J</i>)	0.59 \pm 0.21	0.57 \pm 0.23	14.89	21	<0.05	0.48	1	0.49	1.82	21	0.03
Simpson's diversity index (<i>D</i>)	0.45 \pm 0.18	0.44 \pm 0.22	13.11	21	<0.05	0.02	1	0.9	1.18	21	0.28

All data was analyzed using linear mixed models. *NTA* represents non-target arthropod; *df* represents degrees of freedom; *P* represents corresponding probability

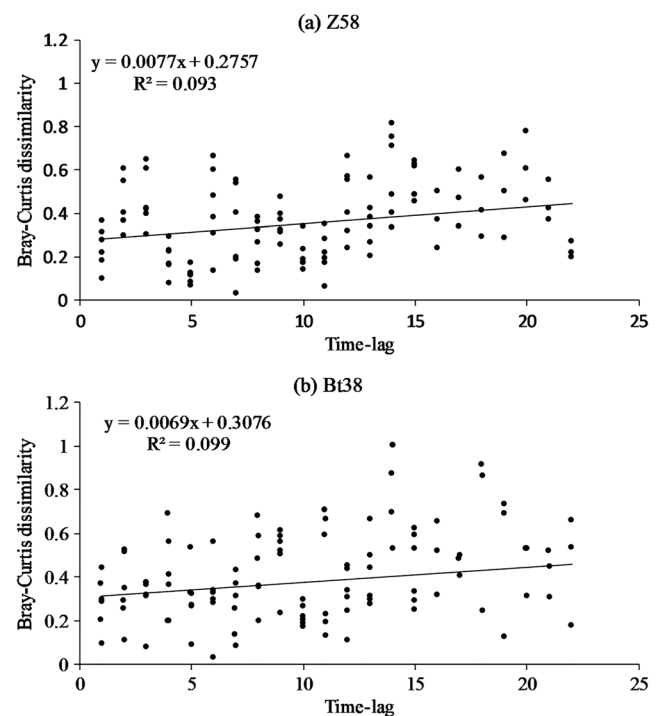
changes in the structure of the NTA community over the 2 years in the Bt corn plots, but the patterns of evolution were similar in both Bt and non-Bt maize plots. Although the dominant species have varied, species richness showed no significant differences between Bt maize and non-Bt maize. We speculated that the change in the Bt maize plots might be due to accompanying changes in microclimate. Climate change itself may play a more important role in population diversity and evenness than Bt maize (Priestley and Brownbridge 2009).

In general, the cultivation of Bt crops was found safe, based on various studies including the insertional impact of transgene and its regulatory elements on plant phenotype and agronomic performance, effect on NTAs, and nutritional impacts on multiple experimental models. Albeit these studies were conducted for limited durations (Mehboob-ur-Rahman et al. 2015), Fernandes et al. (2007) also proved that Bt maize hybrids expressing insecticide proteins Cry1A(b) did not cause differences from their respective non-Bt isogenic hybrids between populations of earwig, lady beetles, minute pirate bug, ground beetles, tiger beetles, and spiders. No uniform effects of Bt maize on the functional guilds of non-target arthropods were found (Wolfenbarger et al. 2008). The 3-year study of the impact of the Bt38 maize strain expressing the Cry1Ac protein on selected groups of NTAs showed the Bt corn had no significantly negative effects on arthropod diversity in the field,

**Fig. 5** Changes in means \pm SE ($n = 6-8$) value of the Bray-Curtis dissimilarity in arthropod diversity between Bt38 and Z58 maize in China

and although *H*, *J*, and *D* varied each year, the differences between Bt38 and Z58 were not generally significant (Table 2). The results were consistent with a previous 2-year monitoring study showing that Bt corn expressing the Cry1Ac insecticidal toxin did not affect NTA community in the fields (Guo et al. 2014). Priestley and Brownbridge (2009) also found that species diversity and evenness of NTAs showed no significant difference between Bt maize expressing the Cry1Ab insecticidal toxin and non-Bt maize.

The Bray-Curtis index is a measure of dissimilarity between two samples, making it suitable to investigate the impact of Bt corn on NTAs and their dissimilarities over time. Potential changes in the structure of the NTA communities due to Cry1Ac toxin exposure were analyzed using the Bray-Curtis dissimilarity (Zhang et al. 2011). The 3-year findings (Fig. 5) were similar to the report showing that there was

**Fig. 6** Time lag analysis of NTA community dynamics in Z58 (a) and Bt38 (b) maize in China

no significant relationship between Bray-Curtis dissimilarity values and elapsed time during the whole study period, indicating that Cry1Ac toxin exposure did not significantly changed NTA diversity in Bt maize plots as compared to non-Bt maize plots (Guo et al. 2014).

Non-target arthropods are selected based on criteria that they are sufficiently abundant in the crop of interest, exhibit low mobility, and possess a clear path of exposure (Romeis et al. 2013). Most studies of the impact of Bt maize on arthropods have found Bt maize that had no negative impact on non-target insects, natural enemies, or arthropod diversity (Devos et al. 2012; Lumbierres et al. 2012). However, these studies were mostly short term, and the lack of long-term data has made these results controversial. Overall, the results of our multi-year study indicate that the effects of transgenic Bt38 maize expressing the Cry1Ac protein on arthropods seem to be relatively minor. The few differences in taxa that may occur across geographies are not barriers to data transportability but require suitable consideration in the context of problem formulation in the environmental risk assessments (Ahmad et al. 2016). With the planting area of Bt maize increasing sharply around the world (James 2018), long-term systematic studies are helpful to confirm the lack of ecological impact of Bt maize on non-target arthropods.

Author contribution Conceived and designed the experiments: YJX, XHD, WPS. Performed the experiments: YJX, ZFQ, MYF, YY, LZ, YW, YXZ. Analyzed the data: YJX. Contributed reagents/materials/analysis tools: YJX, ZFQ, MYF, YW, XHD, SQT, WPS. Wrote the paper: YJX, WPS.

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References

- Ahmad A, Negri I, Oliveira W, Brown C, Asiimwe P, Sammons B, Horak M, Jiang C, Carson D (2016) Transportable data from non-target arthropod field studies for the environmental risk assessment of genetically modified maize expressing an insecticidal double-stranded RNA. *Transgenic Res* 25:1–17
- Arias-Martín M, García M, Castanera P, Ortego F, Farinos GP (2018) Farm-scale evaluation of the impact of Cry1Ab Bt maize on canopy nontarget arthropods: a 3-year study. *Insect Sci* 25:87–98
- Arpaia S, Baldacchino F, Bosi S, Burgio G, Errico S, Magarelli RA, Masetti A, Santorsola S (2018) Evaluation of the potential exposure of butterflies to genetically modified maize pollen in protected areas in Italy. *Insect Sci* 25:549–561
- Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecol Monogr* 27:325–349
- Chen HX, Yang R, Wang Y, Zhang L, Camara I, Dong XH, Liu YQ, Shi WP (2016) Efficacy of Bt maize producing the Cry1Ac protein against two important pests of corn in China. *Environ Sci Pollut Res* 23:21511–21516
- Collins SL, Micheli F, Hartt L (2000) A method to determine rates and patterns of variability in ecological communities. *Oikos* 91:285–293
- Devos Y, Schrijver AD, Clercq PD, Kiss J, Romeis J (2012) Bt-maize event Mon 88017 expressing Cry3Bb1 does not cause harm to non-target organisms. *Transgenic Res* 21:1191–1214
- Fernandes AO, Faria M, Martinelli S, Schmidt F, Carvalho FV, Moro G (2007) Short-term assessment of bt maize on non-target arthropods in Brazil. *Sci Agric* 64:249–255
- Guo YY, Feng YJ, Ge Y, Tetreau G, Chen XW, Dong XH, Shi WP (2014) The cultivation of Bt corn producing Cry1Ac toxins does not adversely affect non-target arthropods. *PLoS One* 9:e114228
- Guo JF, He KL, Bai SX, Zhang TT, Liu YJ, Wang FX, Wang ZY (2016) Effects of transgenic cry1Ie maize on non-lepidopteran pest abundance, diversity and community composition. *Transgenic Res* 25:761–772
- He KL, Wang ZY, Zhou DR, Wen LP, Song YY, Yao Z (2003) Evaluation of transgenic Bt corn for resistance to the Asian corn borer (Lepidoptera: Pyralidae). *J Econ Entomol* 96:935–940
- James C (2018) Global status of commercialized biotech/GM crops: 2017. ISAAA Brief.53. ISAAA, Ithaca
- Leclerc M, Walker E, Messéan A, Soubeyrand S (2017) Spatial exposure-hazard and landscape models for assessing the impact of GM crops on non-target organisms. *Sci Total Environ* 624:470–479
- Lucht JM (2015) Public acceptance of plant biotechnology and GM crops. *Viruses* 7:4254–4281
- Lumbierres B, Albajes R, Pons X (2012) Positive effect of Cry1Ab-expressing Bt maize on the development and reproduction of the predator *Orius majusculus* under laboratory conditions. *Biol Control* 63:150–156
- Mehboob-ur-Rahman, Shaheen T, Irem S, Zafar Y (2015) Biosafety risk of genetically modified crops containing cry genes. CO2 sequestration, Biofuels and depollution
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MH, Szoecs E, Wagner H (2018) Vegan: community ecology package. R package version 2.4–6. <https://cran.r-project.org/package=vegan>. Accessed 17 Jul 2018
- Pielou EC (1966) The measurement of diversity in different types of biological collections. *J Theor Biol* 13:131–144
- Priestley AL, Brownbridge M (2009) Field trials to evaluate effects of Bt-transgenic silage corn expressing the Cry1Ab insecticidal toxin on non-target soil arthropods in northern New England, USA. *Transgenic Res* 18:425–443
- R Core Team (2017) R: a language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. URL <https://www.R-project.org/>. Accessed 17 Jul 2018
- Romeis J, Raybould A, Bigler F, Candolfi MP, Hellmich RL, Huesing JE, Shelton AM (2013) Deriving criteria to select arthropod species for laboratory tests to assess the ecological risks from cultivating arthropod-resistant genetically engineered crops. *Chemosphere* 90:901–909
- Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Illinois Press, Urbana
- Simpson EH (1949) Measurement of diversity. *Nature* 163:688
- Simpson BB, Cracraft J (1995) Systematics: the science of biodiversity. *BioScience* 45(10):670–672
- Toschki A, Hothorn LA, Ross-Nickoll M (2007) Effects of cultivation of genetically modified Bt maize on epigenic arthropods (Araneae; Carabidae). *Environ Entomol* 36:967–981
- Wolfenbarger LL, Naranjo SE, Lundgren JG, Bitzer RJ, Watrud LS (2008) Bt crop effects on functional guilds of non-target arthropods: a meta-analysis. *PLoS One* 3:e2118
- Yoshioka PM (2008) Misidentification of the Bray-Curtis similarity index. *Mar Ecol Prog Ser* 368:309–310
- Zhang B, Chen M, Zhang XF, Luan HH, Tian YC, Su XH (2011) Expressing of Bt-Cry3A in transgenic *Populus alba* × *P. glandulosa* and its effects on target and non-target pests and the arthropod community. *Transgenic Res* 20:523–532