Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services

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Over the past 16 years, vast plantings of transgenic crops producing insecticidal proteins from the bacterium Bacillus thuringiensis (Bt) have helped to control several major insect pests1–3 and reduce the need for insecticide sprays4–6. Because broad-spectrum insecticides kill arthropod natural enemies that provide biological control of pests, the decrease in use of insecticide sprays associated with Bt crops could enhance biocontrol services7–12. However, this hypothesis has not been tested in terms of long-term landscape-level impacts13. On the basis of data from 1990 to 2010 at 36 sites in six provinces of northern China, we show here a marked increase in abundance of three types of generalist arthropod predators (ladybirds, lacewings and spiders) and a decreased abundance of aphid pests associated with widespread adoption of Bt cotton and reduced insecticide sprays in this crop. We also found evidence that the predators might provide additional biocontrol services spilling over from Bt cotton fields onto neighbouring crops (maize, peanut and soybean). Our work extends results from general studies evaluating ecological effects of Bt crops1–6,12,13 by demonstrating that such crops can promote biocontrol services in agricultural landscapes.

Biological control is a valuable ecosystem service14,15, but increasingly intensive farming strongly influences the populations of natural enemies and the biocontrol services they provide16–18. However, landscape biodiversity management and restricted use of pesticides may enhance biocontrol services in agro-ecosystems and could thus favour the development of sustainable farming19. Genetically engineered crops that express δ-endotoxins (Cry proteins) from Bacillus thuringiensis (Bt) have been increasingly implemented by farmers in many countries since 1996, and more than 6.6 × 107 ha of Bt crops were planted worldwide in 2011 (ref. 19). Bt crops have successfully controlled several major insect pests2,4,12,15 and led to a drastic decrease in insecticide use on these crops2,6,12. Because insecticide applications have been gradually reduced in Bt crops, their widespread adoption may benefit natural enemies and may therefore potentially enhance associated ecosystem services such as the control of arthropod pests10–12. This last point has not yet been documented, especially with regard to the long-term landscape-level impacts10.

From the 1970s, insecticides were applied extensively to control cotton bollworm (CBW), Helicoverpa armigera, the most serious insect pest on conventional cotton in China. However, control became almost impossible in the early 1990s because the pest became resistant to most insecticides, and unprecedented outbreaks in 1992 led to a wide overuse of insecticides. Consequently, in 1993, the Chinese government requested systematic insecticide applications in wheat crops for the control of the first-generation CBW; that is, before the following generations colonized cotton crops20. Although insecticide use decreased in cotton, this measure was not sustainable because insecticide applications were increased on wheat crops, resulting in both higher costs and environmental pollution. Bt cotton was therefore approved in 1997 for commercial use to control CBW, and it became the Chinese government’s key measure against this cotton pest. It was rapidly planted on a large scale, rising to 2.4 × 106 ha by 2011 (more than 95% of the cotton crop in northern China). It managed CBW effectively, which led to decreased insecticide use on this pest21.

The widespread adoption of Bt cotton may have favoured an increase in generalist natural enemy populations and promoted their associated biocontrol services. We therefore performed two assessments: first, whether implementing Bt cotton on a large scale induced an increase in populations of three groups of key generalist predators in China (ladybirds, lacewings and spiders) in both Bt cotton and three common neighbouring crops, namely maize, peanut and soybean; and second, whether this trend resulted in increased biocontrol services in agricultural landscapes in China. Aphids were selected as a pest model because they are common prey for generalist predators. During 1990–2011, research was conducted in six major cotton-growing provinces (Henan, Hebei, Shandong, Shanxi, Anhui and Jiangsu) in northern China, where about 2.6 × 106 ha of cotton and 3.3 × 107 ha of other crops (notably maize, peanut and soybean) are cultivated annually by more than ten million small-scale farmers.

Figure 1 | Population densities of predators and aphids on cotton with different management regimes at Langfang experimental station (2001–2011). a, Predators. b, Aphids. The blue and red lines indicate Bt cotton and non-Bt cotton without insecticide sprays, respectively; the green line represents non-Bt cotton with CBW insecticide sprays (chemical control).

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Predators and cotton aphids were sampled from 2001 to 2011 in Bt and non-Bt cotton plots at Langfang experimental station in Hebei province. No significant differences were found for predator ($P = 0.341$) and aphid ($P = 0.555$) abundances between Bt cotton and non-Bt cotton with similar management methods; that is, without application of insecticides (Fig. 1a, b and Supplementary Table 1a, b). However, predator abundance was significantly lower and aphid abundance was significantly higher in plots treated with insecticides for CBW management in comparison with insecticide-free plots ($P < 0.001$) (Fig. 1a, b and Supplementary Table 1a, b), although it varied over years (significant interactions between insecticide application and year). Bt cotton does not itself affect predator and aphid population levels$^{10,22}$, and generalist predators are clearly susceptible to broad-spectrum insecticides (such as synthetic pyrethroids) used against CBW. Thereafter, insecticide-induced aphid resurgence usually occurs with widespread applications of insecticides.

Predator abundance and insecticide use in cotton were monitored in 36 locations throughout northern China during 1990–2010 (Fig. 2a and Supplementary Table 2). Predator population levels gradually increased over that period, and relatively high population levels were always observed after Bt cotton was implemented in 1997 (Fig. 2b). In 14 selected locations, all three major groups of predators (ladybirds, lacewings and spiders) showed an increasing trend similar to that of the whole predator complex (Fig. 2b). Insecticide use patterns also changed greatly with Bt cotton implementation. After the introduction of Bt cotton, the number of insecticide sprays against CBW (and other insect pests in general), mainly pyrethroid and organophosphate insecticides (Supplementary Table 3), which have multiple negative effects on natural enemies$^{17}$, was lower than during the pre-Bt cotton period, namely 1990–1996 (Fig. 2c). Moreover, predator population level and number of insecticide sprays were positively and negatively related to Bt cotton planting proportions, respectively ($P < 0.001$; Supplementary Fig. 1a, b), and indicated the effect of its large-scale adoption on the predator population trend. Regression analyses showed that fewer insecticide sprays against CBW and all insect pests were correlated to a great extent with an increase in predator populations in northern China ($P < 0.001$) (Fig. 2d, e). The results were consistent in the six provinces, and insecticide use against CBW was a driving factor for predator population level in the cotton agroecosystem (all $P < 0.05$; Supplementary Table 4).

Cotton aphid abundance was surveyed in 24 locations from 1990 to 2010 (Supplementary Table 2) to assess the biocontrol services provided by generalist predators. Linear regression analyses showed that increasing generalist predator populations were correlated with decreasing aphid abundance in northern China in general ($P < 0.001$; Fig. 3a) and in all provinces except Shanxi (Supplementary Fig. 2a–e). During the three main periods studied—that is, without Bt cotton, with less than 90% and more than 90% of Bt cotton planting in the landscapes—aphid populations decreased significantly ($P < 0.001$; Fig. 3b). In addition, aphid population was negatively related to the proportion of Bt cotton planted ($P = 0.003$; Supplementary Fig. 3). Exclusion cage trials in 2010 and 2011 at Langfang and Xinxing experimental stations (in Figure 2 | Relationships between predator population density and number of insecticide sprays on cotton in northern China (1990–2010). a, Survey locations, indicated by red dots. b, Predator population density on cotton in commercial fields in 36 locations (each point represents one-year data; the red arrow indicates the beginning of Bt cotton use). Inset: population abundance of ladybirds (blue), spiders (red) and lacewings (green), collected from 14 locations. c, Number of insecticide sprays for CBW (grey points) and all insect pests (black points) on cotton; each point represents one-year data. d, Linear relationship between total number of insecticide applications, determined by pooling all treatments against all the insect pests on cotton (x), and the predator abundance (y) in cotton ($y = -1.69x + 30.63, F_{1,119} = 71.19, R^2 = 0.79, P < 0.0001$). e, Linear relationship between number of insecticide applications for CBW only (x) and predator abundance (y) ($y = -1.11x + 16.03, F_{1,119} = 137.32, R^2 = 0.88, P < 0.0001$). The data in d and e are replotted from b and c. All error bars show s.e.m.
Hebei and Henan provinces, respectively) further demonstrated the significant effects of predators on aphid population growth in cotton fields (Supplementary Fig. 4a, b). As the cotton aphid populations declined, an invasive whitefly in cotton, *Bemisia tabaci*, probably served as an alternative prey for the increasing predator populations.

All these results indicate that the widespread adoption of Bt cotton ultimately promotes biocontrol services in the agroecosystem because decreased insecticide use leads to an increase in predator populations. Broadly speaking, measures that preserve predators in cotton fields greatly help to control aphid populations; for example, when insecticide applications in wheat were requested by the Chinese government (1993–1996) to prevent CBW outbreaks in cotton (see above), it led to a decreasing trend in aphid abundance (Fig. 3b).

Predator abundance was also monitored from 2001 to 2011 in three neighbouring crops: maize, peanut and soybean at Langfang experimental station. There was a positive relationship between predator abundance in cotton and soybean (*P* = 0.019; Fig. 4a), as well as between cotton and peanut (marginally significant, *P* = 0.075; Fig. 4b). We observed a similar trend in maize but it was not significant (*P* = 0.216; Fig. 4c). The increased predator abundance in maize was linked to a decrease in aphid pest abundance in that particular crop (marginally significant, *P* = 0.061; Fig. 4d).

Biocontrol services are important components in agro-ecosystems and could lead to the development of sustainable agriculture. In conventional agricultural practices, insecticides are frequently used to control targeted pests, but they can lead to outbreaks of secondary pests by suppressing their natural enemies. This so-called insecticide-induced resurgence was first reported for cotton aphid in the 1970s and was regarded as a key factor leading to population outbreaks of this pest in China. Our work demonstrates the importance of natural enemies in the long-term suppression of the cotton aphid. The widespread adoption of Bt cotton, as a sustainable measure to reduce insecticide use, has indirectly promoted generalist predator abundance in Bt cotton fields but also to a smaller extent in three common adjacent crops in northern China. Bt crops therefore might enhance biocontrol services in agricultural landscapes through an increased abundance of generalist natural enemies. This study provides key information on long-term landscape-level ecological effects of Bt crops as well as useful insights, for example into the management of pest resurgence problems reported for many pests worldwide.

Generalist predators usually have great dispersal ability and can rely on various food sources. Hence, not only can they synchronously attack different insect pests in one field, but they can also colonize different habitats in different seasons. Furthermore, some habitat management measures, such as inter-planting different crops or wild plants, have been adopted to provide resources such as food supply or shelter for natural enemies, thus increasing conservation biological control in adjacent fields. We have demonstrated that decreasing insecticide application, through widespread Bt cotton plantings, sustained generalist predators and helped to suppress aphid populations in this.
crop. Large-scale insecticide reduction is the key driver in such processes (for example see the period 1993–1996, during which insecticide decrease favoured an increase in predator populations and a decline of aphid populations). Higher generalist predator population levels in Bt cotton lead to lower insect pest levels in the crop, and these predators might provide additional biocontrol services spilling over from cotton fields onto neighbouring crops, although further work should be performed to document this last point. Broadly speaking, the deployment of Bt crops may favour biocontrol services and enhance economic benefits not only in Bt crop fields but also in the whole agricultural landscape. Field studies indicated that Bt crops protected natural enemies in comparison with non-Bt crops, which rely on conventional insecticides\textsuperscript{27,28}. Our present study, demonstrating that biocontrol services are potentially provided by Bt crops throughout the agricultural landscape, may offer new options in developing conservation biological control measures at the landscape level.

Critical concerns about the ecological risk assessment of transgenic crops still remain, especially on a large scale\textsuperscript{29}. The present study confirms no negative effects of one Bt crop, Bt cotton, on generalist predators in agricultural landscapes in China. More particularly, we have demonstrated a marked increase in generalist predator population levels and associated biocontrol services linked to decreased insecticide use owing to the widespread adoption of the Bt crop. Our work provides a comprehensive, long-term and large-scale assessment of the possible ecological and agricultural effects of transgenic crops.

METHODS SUMMARY

The study was based on large-scale surveys of predator and cotton aphid populations in cotton fields of northern China from 1990 to 2010 and on experiments and surveys that were performed at Langfang experimental station of the Chinese Academy of Agricultural Science (CAAS) during the period 2001–2011. The surveys and experiments focused on three major generalist predator groups (ladybirds, lacewings and spiders) and on aphid pests in cotton and in three common cotton-neighbouring crops, namely maize, peanut and soybean. At the CAAS, we first assessed how cultural practices could affect predator and aphid populations in the long term in cotton fields; cotton plots were established every year and the abundance of predators and cotton aphids was surveyed in three different plot types: Bt cotton, non-Bt cotton and non-Bt cotton with insecticide. Second, we determined the impact of predators on aphid population in cotton by means of exclusion cage trials. Third, we evaluated the impact of implementing Bt crop on the abundance of nontarget arthropod natural enemies.

Large-scale surveys were conducted in six provinces in northern China (36 locations, 10–20 fields per location) to evaluate the impact of insecticide applications on the abundance of predators and aphids in cotton fields. We tested, first, the relationship between predator abundance and insecticide use during the period 1990–2010 (that is, including the period before and during the widespread adoption of Bt cotton by farmers), and second, how cotton aphid density was related to predator abundance during the same period.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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METHODS
Aphid pests and predator complex considered in the study. In northern China, several aphid species are reported as pests on cotton, maize, peanut and soybean. *Aphis gossypii* Glover (cotton aphid) is the main aphid pest in cotton fields in northern China where there are two key biotypes (called seedling and summer aphids, respectively). Only the summer aphid, which colonizes fields from early July to late August, is considered a major cotton pest\(^{20,21}\). The seedling aphid is controlled by insecticide treatments applied on the seeds; these compounds do not last long enough within plants to provide control of the summer aphid\(^{22}\). *Rhopalosiphum maidis* Fitch, *R. padi* Linnaeus and *Stilobium avenue* Fabrini are the three dominant aphid species on maize\(^{23}\), and *A. glycines* Matsushita and *A. craccivora* Koch are the main aphid species on soybean and peanut, respectively\(^{24}\). In our study, the above aphid species were considered in assessing the biocontrol services provided by generalist predators.

In northern China, there are three dominant groups of generalist predators in cotton field (more than 90% of all the predators)\(^{25}\): ladybirds, lacewings and spiders. In our study we therefore focused on a predator complex composed of ladybirds (*Propylea japonica* Thunberg, *Harmonia axyridis* Pallas, *Coccinella septempunctata* L. and *Adonia variegata* Gozei), lacewings (*Chrysopa septem-punctata* Wsmæa, *Chrysopa semiola* Tjder and *Chrysopa formosa* Brauer) and spiders (*Erigonidium graminicolum* Sundevall, *Misumenopus triscupulata* Fabrini; *Pterostichus t-insignita* Boes. et Str.); these compose the most common predators in agricultural landscape of that region. These groups of predators are also common in maize, peanut and soybean fields and were thus also considered as a predator complex for these three crops\(^{12,26}\).

Impact of agricultural practices on predator and cotton aphid populations. Survey experiments were conducted from 2001 to 2011 at Langfang experimental station (39.53° N, 116.70° E), Chinese Academy of Agricultural Sciences (CAAS), Hebei province, China. Fifteen cotton plots (400 m² each) were established every year and were managed with agronomic practices that are standard in northern China. A randomized block design with three replicates was used, which included two Bt and two conventional cotton varieties. One Bt cotton variety expressing Cry1Ac (NuCOTN33B) and another Bt cotton varieties expressing Cry1A (SGK321) were supplied by Monsanto Co. and the Biotechnology Research Institute, CAAS, respectively. Two conventional cotton varieties (Shiyuan321 and Zhong12) were obtained from the Institute of Plant Protection, CAAS. Shiyuan321 was the non-transgenic isolate of SGK321. Every year, the trial consisted of three treatments: Bt cotton and non-Bt cotton plots without insecticide, and non-Bt cotton (one variety, Zhong12) plots with insecticides. \(\beta\)-Cypermethrin (pyrethroid) and phoxim (organophosphate) were used when insecticides were applied. The choice of these two insecticides, and their frequency of application, were both based on management guidelines for CBW and all insect pests for each province and the whole of northern China in the 1990–2010 data set. Simple linear models were used to assess the relationship between aphid density (log-transformed) and predator abundance from early July to late August (in the 1990–2010 data set) for each province and for the whole of northern China. Linear regression analyses were used to assess the relationship between predator abundance and aphid abundance (log-transformed) with Bt cotton planting proportions. In this analysis, the mean abundances of predators and aphids during 1990–1996 were included as the data when the Bt cotton planting proportion was 0.

To evaluate the impact of the predators on cotton aphid population further, exclusion cage trials were conducted in 2010 and 2011 at Langfang experimental station and Xingxiang experimental station of CAAS (Henan Province, 35.09° N, 113.48° E). This trial included a caged treatment and an open-field treatment as control. The cage was a cylinder of 2 m wide by 1.5 m high and made from the insect mesh net, which allowed the emigration and immigration of alate aphids and their parasitoids, but blocked the predators\(^{27}\). Ten cotton plants were covered in each cage. This trial began in July, when almost only apterous aphids were in the field\(^{28}\) and was limited to 15 days to prevent the appearance of alate aphids in the cage\(^{1,27,28}\). At each site, cage treatments with three or four replicates were established when aphid density reached an average of 2 individuals per plant in 2010 and 20 individuals per plant in 2011. We recorded the aphid abundance 15 days after treatment. Meanwhile, predator densities were surveyed three times, on day 0, day 5 and day 10, in ten randomly selected cotton plants in open field during the whole trial. The aphid abundance in caged and open plants was compared by one-way ANOVA followed by a post-hoc LSD test. Before analysis, the data for aphid abundance were log-transformed.

Impact of Bt cotton adoption on populations of predators in neighbouring crops. Population dynamics of the predator complex were monitored in cotton, soybean, peanut and maize field plots from 2001 to 2011 (except for maize, which was monitored during 2001–2003 and 2008–2011, and soybean, which was monitored during 2001–2006 and 2008–2011) at Langfang experimental station. Every year, a total of nine field plots (400 m² each) were established for each crop type and they were managed in the same way, applying the same fertilizers and irrigation treatment, free of any pesticide. A randomized block design with three replicates for each crop type was used. One Bt cotton variety, SGK321, was supplied by the Biotechnology Research Institute (CAAS); the maize (var. Shengshi29), soybean (var. Zhonghuang13) and peanut (var. Huayu16) were provided by Langfang experimental station (CAAS), the Institute of Crop Sciences (CAAS) and the Shandong Peanut Research Institute, respectively. The abundance of predators was recorded in the four different crops (Bt cotton, maize, peanut and soybean) every four or five days from mid-June to late August. At each sampling date, 100 plants in five random spots per plot were visually inspected and all predators were recorded. Linear regression analyses were used to assess the relationship between seasonal density of predators on cotton and soybean/peanut (data set covering the 2001–2011 period) and maize (data set covering the 2001–2003 and 2008–2011 periods).

The maize plots, maize aphids were also recorded because they are well known as the main pests on maize in northern China. Population levels of aphids on soybean and peanut crops at Langfang experimental station were very low during the course of our study and therefore the data could not be considered in the framework of the study. A simple linear model was used to assess the relationship between aphid abundance (log-transformed) and predator abundance on maize.


