



## Mirid Bug Outbreaks in Multiple Crops Correlated with Wide-Scale Adoption of Bt Cotton in China

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not account for near modern deep-water ventilation ages observed during the ACR/Bølling-Allerød (Fig. 2E). This would suggest that either the export rate or ocean-atmosphere equilibration of one or both of the main Atlantic deep-water end-members (North Atlantic and/or Antarctic) increased during the ACR/Bølling-Allerød.

Our results demonstrate the existence before HS1 of an exceptionally aged abyssal carbon reservoir that could have substantially contributed to the sequestration of CO<sub>2</sub> in the deep sea during the last glacial period. These results also underline the potential importance of the combined effects of changing Antarctic sea ice, wind forcing, and abyssal stratification on the deglacial rise of CO<sub>2</sub>. As illustrated schematically in Fig. 4, we envisage that although the meridional extent of Antarctic sea ice should influence the efficacy of the westerly wind stress that can effectively be applied to the Antarctic Circumpolar Current (ACC) to drive the upwelling of the densest classes of CDW in the Southern Ocean, the impact of upwelling on atmospheric CO<sub>2</sub> ( $\Delta^{14}\text{C}_{\text{atm}}$  and  $\delta^{13}\text{CO}_2$ ) might ultimately be determined by changes in the potential  $P_{\text{CO}_2}$  and sequestration age of the CDW that is brought to the surface. Thus, the deglacial trends in atmospheric CO<sub>2</sub>,  $\delta^{13}\text{CO}_2$ , and  $\Delta^{14}\text{C}_{\text{atm}}$  may have been interrupted during the ACR/Bølling-Allerød at least partly as a result of a pronounced increase in the ventilation of CDW brought to the surface Southern Ocean at this time (Figs. 2D and 4D). In contrast, with the preponderance of exceptionally aged CDW during HS1 and the YD [and with Antarctic sea ice already pulled back from its maximal

meridional extent (Fig. 4C)], high potential  $P_{\text{CO}_2}$  water would be brought to the surface Southern Ocean instead. The result would have been to greatly enhance the release of <sup>14</sup>C-depleted CO<sub>2</sub> to the atmosphere, as well as the export of <sup>14</sup>C-depleted water from the ACC to the Atlantic and Pacific at these times.

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## Mirid Bug Outbreaks in Multiple Crops Correlated with Wide-Scale Adoption of Bt Cotton in China

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Long-term ecological effects of transgenic *Bacillus thuringiensis* (Bt) crops on nontarget pests have received limited attention, more so in diverse small holder-based cropping systems of the developing world. Field trials conducted over 10 years in northern China show that mirid bugs (Heteroptera: Miridae) have progressively increased population sizes and acquired pest status in cotton and multiple other crops, in association with a regional increase in Bt cotton adoption. More specifically, our analyses show that Bt cotton has become a source of mirid bugs and that their population increases are related to drops in insecticide use in this crop. Hence, alterations of pest management regimes in Bt cotton could be responsible for the appearance and subsequent spread of nontarget pests at an agro-landscape level.

Genetically engineered crops that express  $\delta$ -endotoxins (Cry proteins) from *Bacillus thuringiensis* (Bt) can successfully control several insect pests. The adoption of Bt crops increases yield and causes vast reductions in insecticide use (1–5). With Bt crops presently adopted

in over 20 countries (6), the ecological risks of their commercial cultivation have received considerable scientific scrutiny (7–11). In China, Bt cotton was approved in 1997 for commercial use to control cotton bollworm, *Helicoverpa armigera*, and has steadily been adopted by the bulk of Chinese

cotton growers (i.e., presently 95% adoption in northern China). Bt cotton controls *H. armigera* larvae very effectively and acts as a dead-end trap crop for regional populations of this pest in local agricultural landscapes (12); that is, a large percentage of the pest moths lay their eggs in cotton, where the hatching larvae are killed and do not subsequently infest other crops as adults. Hence, Bt cotton controls a key target pest not only within cotton fields but also on multiple other non-Bt host crops (i.e., corn, peanuts, soybeans, and vegetables), reducing the overall need for insecticide sprays (3, 12). Nevertheless, long-term impacts of Bt cotton on nontarget arthropods, such as polyphagous insect pests, in local agro-ecosystems remain to be quantified (13–15).

Mirid bugs (Heteroptera: Miridae) are herbivores in a broad range of cultivated plants, including

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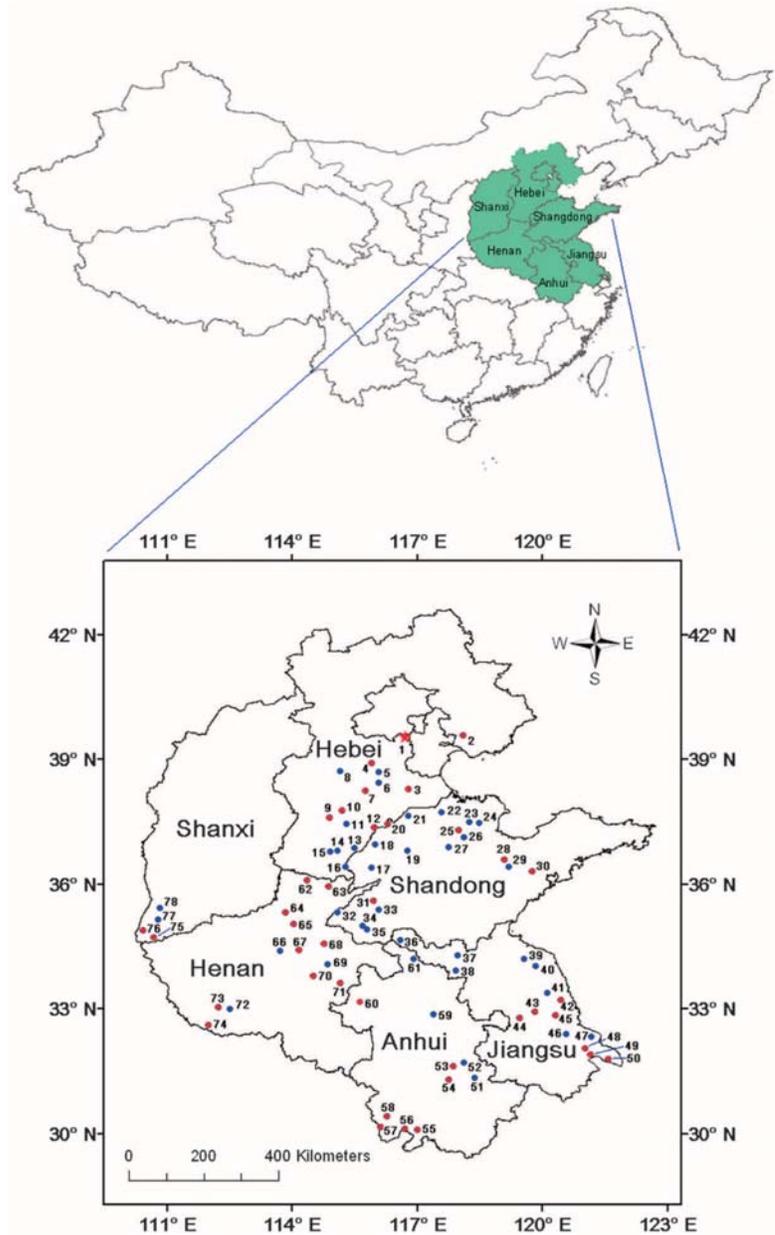
cotton, cereals, vegetables, and fruit crops. In China, mirid bugs have historically been considered occasional or minor pests in most crops, occurring at relatively low population densities and only sporadically requiring pest management intervention (16). Nevertheless, mirid bugs can easily attain outbreak densities, switch host crops, or experience geographic spread because of their environmental adaptability (16–18), high population growth rate (16, 17), and strong dispersal capacity (19, 20). In this study, we determined whether mirid bug outbreaks are more likely to occur in Bt cotton than in conventional cotton and to what extent these insects could cause unintended ecological impacts in the broader agro-ecosystem (21). Research was conducted in six major cotton-growing provinces (i.e., Henan, Hebei, Jiangsu, Anhui, Shandong, and Shanxi) of northern China (Fig. 1), where 3 million ha of cotton and 26 million ha of alternative crops, potentially susceptible to mirid bug attack, are cultivated annually by >10 million small-scale farmers.

Mirid bugs were sampled from 1998–2009 in Bt and non-Bt cotton plots at the Langfang Experiment Station in Heibei province (21). Mirid bug abundance did not differ between cotton varieties with similar management regimes ( $P > 0.05$ ), and calendar-based insecticide sprays for *H. armigera* control in non-Bt cotton significantly lowered mirid bug infestation densities ( $P < 0.05$ ) (Fig. 2 and table S3). Hence, Bt cotton per se does not affect mirid bug infestation densities, and mirid bug populations prove susceptible to broad-spectrum insecticides for *H. armigera*.

We simultaneously monitored mirid bug abundance and insecticide use in cotton at 38 locations throughout the study region during 1997–2008 and 1992–2008, respectively (21). Mirid bug population levels gradually increased over time and were significantly related to Bt cotton planting proportion ( $P < 0.05$ ) (Fig. 3). Insecticide use patterns also changed with Bt cotton adoption (fig. S1). After introduction of Bt cotton, the number of insecticide sprays against *H. armigera* and all insect pests was evidently lower than during 1992–1996. On the other hand, the number of sprays against mirid bugs increased over time, in line with Bt cotton planting proportion (fig. S1). Meanwhile, *H. armigera* insecticide use was a highly explanatory variable for annual mirid bug population levels and insecticide use for mirid bug control ( $P < 0.05$ ) (Table 1).

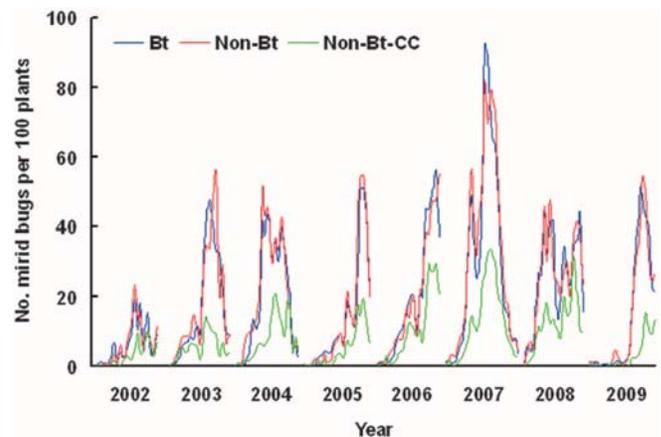
Additionally, mirid bug infestations were recorded in alternative host crops: Chinese date, grapes, apple, peach, and pear (21). Mirid bug infestation severity increased in alternative host crops, and respective infestation severities were significantly correlated with regional proportion of Bt cotton planted ( $P < 0.05$ ) (Fig. 4).

Over the study period, mirid bugs gradually increased population densities and damage in cotton and multiple other crops. Mirid bug attraction to flowering plants and associated seasonal host alternation partially explains the observed pattern



**Fig. 1.** Survey locations in northern China. Mirid bug population dynamics on Bt and non-Bt cotton were monitored at Langfang site (star). Population densities were surveyed on cotton at 38 sites (red), and infestation densities were determined on other host crops at 77 sites (red and blue).

**Fig. 2.** Mirid bug population dynamics in Bt and non-Bt cotton with different management regimes from 2002–2009. Bt and Non-Bt indicate Bt cotton and non-Bt cotton, respectively, without insecticide sprays, whereas Non-Bt-CC represents non-Bt cotton with *H. armigera* insecticide sprays.



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(16, 22). In mid- to late June, mirid bugs largely move from early-season host plants to crop fields, where they build up their initial populations. Results of 2006–2009 field-plot trials indicate that mirid bugs greatly prefer cotton over other major

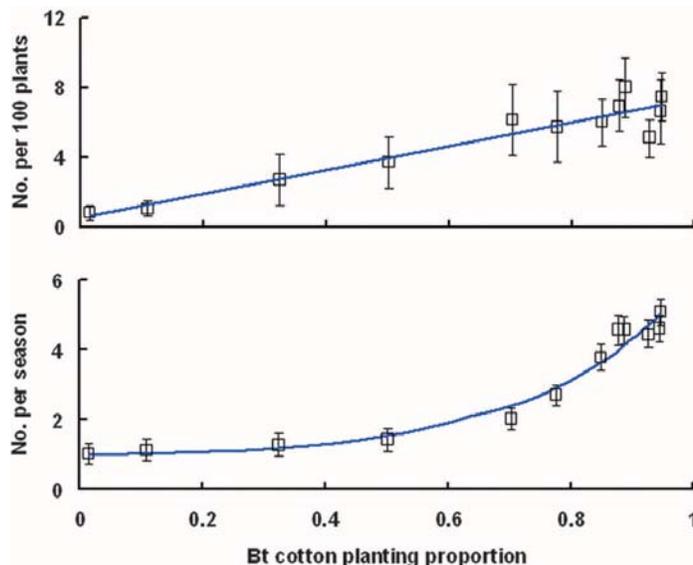
host crops in mid- to late June (fig. S2), because cotton is locally one of few flowering host crops during this time period. Before Bt cotton adoption, broad-spectrum *H. armigera* insecticide use reduced early mirid bug populations, with cotton

acting as a dead-end trap crop. Current absence of insecticide sprays in Bt cotton permits unrestrained mirid bug population buildup and subsequent (active) spread or (passive) spillover to a multitude of other (flowering) crops. Hence, a reduction in insecticide use for *H. armigera* control in Bt cotton correlates with mirid bug outbreaks in cotton and various fruit crops in the broader agro-landscape.

Most polyphagous insects exhibit clear preferences for one or few host plants and may seasonally concentrate in patches of these plants. Consequently, management actions in these patches can greatly determine population dynamics of such insects at the landscape level (23). Our work shows that a drop in insecticide use in Bt cotton fields leads to a reversal of the ecological role of cotton: from being a sink for mirid bugs in conventional systems to being an actual source for these pests in Bt cotton-growing systems. This perspective should be instrumental in developing regionwide management strategies for these polyphagous pests in northern China and elsewhere in the world.

Pest resurgence and replacement are usually ascribed to alterations in pest management re-

**Fig. 3.** Association between mirid bug population density (top) or number of mirid bug insecticide sprays (bottom) and Bt cotton planting proportion in northern China during 1997–2008. Linear model for population density:  $y = 6.81x + 0.54$ ,  $F_{1,10} = 88.65$ ,  $P < 0.0001$ , coefficient of determination ( $R^2$ ) = 0.90. Nonlinear model for number of mirid bug insecticide sprays:  $y = 0.89 + 0.08 \cdot \exp(4.21x)$ ,  $F_{2,9} = 126.46$ ,  $P < 0.0001$ ,  $R^2 = 0.97$ .

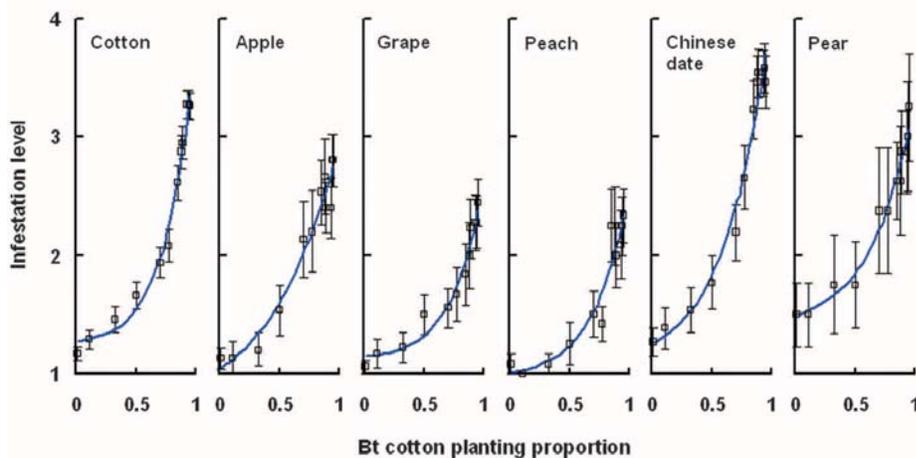


**Table 1.** Multivariate assessment of determinants of mirid bug population density and insecticide spray frequency in northern China during 1997–2008. For stepwise regression, only variables with significance  $P < 0.05$  were

entered into the analysis. “Other insecticide sprays” target insect pests different from *H. armigera* or mirid bugs.  $r$ , regression coefficient.  $R^2$ , coefficient of determination. A slash indicates no analysis.

Parameters	Mirid bug population density						Number of mirid bug insecticide sprays					
	Single regression			Stepwise regression			Single regression			Stepwise regression		
	$r$	$R^2$	$P$	$r$	$R^2$	$P$	$r$	$R^2$	$P$	$r$	$R^2$	$P$
Total number of insecticide sprays	-0.2093	0.51	0.0095				/			/		/
Number of <i>H. armigera</i> insecticide sprays	-0.1079	0.87	<0.0001	-0.1079	0.87	<0.0001	-0.7214	0.84	<0.0001	-0.7214	0.84	<0.0001
Number of other insecticide sprays	+0.1361	0.01	0.7131				-1.8505	0.06	0.4570			
Temperature	-0.0921	0.04	0.5242				-0.4116	0.02	0.6773			
Rainfall	-0.0001	<0.01	0.8956				-0.0005	<0.01	0.9487			

**Fig. 4.** Association between mirid bug infestation severity in either cotton or key fruit crops and Bt cotton planting proportion. The measure of mirid bug infestation was assigned a score ranging from 1 (no infestation) to 5 (extreme infestation). Nonlinear model for cotton,  $y = 1.23 + 0.04 \cdot \exp(4.24x)$  ( $F_{2,9} = 346.40$ ,  $R^2 = 0.99$ ,  $P < 0.0001$ ); apple,  $y = 0.64 + 0.40 \cdot \exp(1.76x)$  ( $F_{2,9} = 107.77$ ,  $R^2 = 0.96$ ,  $P < 0.0001$ ); grape,  $y = 1.12 + 0.02 \cdot \exp(4.15x)$  ( $F_{2,9} = 118.05$ ,  $R^2 = 0.96$ ,  $P < 0.0001$ ); peach,  $y = 0.97 + 0.04 \cdot \exp(3.61x)$  ( $F_{2,9} = 46.07$ ,  $R^2 = 0.91$ ,  $P < 0.0001$ ); Chinese date,  $y = 1.06 + 0.20 \cdot \exp(2.74x)$  ( $F_{2,9} = 166.88$ ,  $R^2 = 0.97$ ,  $P < 0.0001$ ); and pear,  $y = 1.39 + 0.11 \cdot \exp(2.88x)$  ( $F_{2,9} = 153.39$ ,  $R^2 = 0.97$ ,  $P < 0.0001$ ).



gimes (24). For example, cotton aphid, *Aphis gossypii*, evolved as a primary pest of cotton in the mid-1970s because of intensive insecticide use for *H. armigera* control (25, 26) but currently occurs at low population densities in Bt cotton (27). In a similar fashion, mirid bugs were previously suppressed by *H. armigera* insecticide use and only acquired pest status in cotton and other crops after Bt cotton adoption. Hence, areawide cultivation of transgenic crops may bring various (direct and indirect) effects on ecological status of different organisms, which should be assessed or anticipated in a comprehensive fashion.

In many parts of the world, transgenic crops such as Bt crops have come to dominate agricultural landscapes, and their landscape-level impact has been quantified on target pests (12, 28), nontarget organisms such as natural enemies (7, 29, 30), or charismatic species such as the monarch butterfly *Danaus plexippus* (31). However, few studies have described the impact of transgenic crops on nontarget insect pests (2, 13) or, more specifically, assessed their landscape-level effects (15, 32, 33). This study confirms reports of a landscape-level emergence of nontarget pests with the adoption of Bt crops resulting from reductions in insecticide applications. Our work highlights a critical need to predict landscape-level impacts of transgenic crops on (potentially) pestiferous organisms in future ecological agricultural risk assessment. Such more-comprehensive risk management may be crucial to help advance integrated pest management and ensure sustainability of transgenic technologies.

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#### Supporting Online Material

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Figs. S1 and S2

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## Prion Strain Mutation Determined by Prion Protein Conformational Compatibility and Primary Structure

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Prions are infectious proteins composed of the abnormal disease-causing isoform PrP<sup>Sc</sup>, which induces conformational conversion of the host-encoded normal cellular prion protein PrP<sup>C</sup> to additional PrP<sup>Sc</sup>. The mechanism underlying prion strain mutation in the absence of nucleic acids remains unresolved. Additionally, the frequency of strains causing chronic wasting disease (CWD), a burgeoning prion epidemic of cervids, is unknown. Using susceptible transgenic mice, we identified two prevalent CWD strains with divergent biological properties but composed of PrP<sup>Sc</sup> with indistinguishable biochemical characteristics. Although CWD transmissions indicated stable, independent strain propagation by elk PrP<sup>C</sup>, strain coexistence in the brains of deer and transgenic mice demonstrated unstable strain propagation by deer PrP<sup>C</sup>. The primary structures of deer and elk prion proteins differ at residue 226, which, in concert with PrP<sup>Sc</sup> conformational compatibility, determines prion strain mutation in these cervids.

Prions are protein-based transmissible agents causing lethal, incurable neurodegenerative diseases of mammals, including sheep scrap-

ie, bovine spongiform encephalopathy, human Creutzfeldt-Jakob disease, and chronic wasting disease (CWD), a contagious prion disorder of

cervids (a family of hoofed mammals, including deer and elk). During propagation, PrP<sup>Sc</sup>, the  $\beta$ -sheet-rich disease-associated conformer of the prion protein (PrP), coerces the physiological form, PrP<sup>C</sup>, to adopt the PrP<sup>Sc</sup> conformation. Prions share with nucleic acid-based pathogens the ability to propagate strain information. Although distinct conformers of PrP<sup>Sc</sup> appear to encipher the characteristics of certain strains (1–5), it is unclear how prions mutate and adapt in the absence of nucleic acids. Strain mutation has been reported after interspecies prion transmission—for example, scrapie transmission to rodents (6). Prion mutation may result in increased host range (7), a factor that complicates risk assessments for new hosts.

Although strain diversity has been recorded for sheep, human, and bovine prions, the existence of strains in CWD is unclear (8–10). Polymorphic residue 129 of human PrP and the corresponding elk PrP residue influence prion susceptibility at the level of strain selection (11, 12). PrP primary structures of CWD-susceptible species differ at residue 226, which is glutamic acid (E) in Rocky Mountain elk and glutamine (Q) in other susceptible cervids. To determine CWD strain prevalence and to assess the influence of