



Susceptibility of *Helicoverpa armigera* to two Bt toxins, Cry1Ac and Cry2Ab, in northwestern China: toward developing an IRM strategy

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Abstract

Extensive unofficial planting of Bt cotton expressing Cry1Ac toxin has occurred for the past two decades in northwestern China, and no mandatory refuge policy has been adopted. The status of Cry1Ac susceptibility of *Helicoverpa armigera* in this region has not been routinely monitored, nor has the susceptibility to Cry2Ab cotton which has not been released in China. The susceptibility of *H. armigera* populations to both toxins was assessed in 2014 and 2015 in two contrasting cotton farming systems across the region. Over the 2 years, the response to Cry1Ac of the nine *H. armigera* field populations sampled ranged from 3.16 to 16.94 $\mu\text{g ml}^{-1}$ for LC_{50} and 0.013 to 0.741 $\mu\text{g ml}^{-1}$ for IC_{50} , and the baseline susceptibility of these strains to Cry2Ab ranged from 3.43 to 19.05 $\mu\text{g ml}^{-1}$ for LC_{50} and 0.16 to 3.81 $\mu\text{g ml}^{-1}$ for IC_{50} . There was no significant difference in susceptibility to either Cry1Ac or Cry2Ab between small-holder and broad-acre farming. The susceptibility to Cry1Ac toxin in northwestern China is higher than that in northern China, while there was no difference for Cry2Ab between northwestern China and northern China. With high levels of adoption of Bt cotton and relatively limited natural refuge for *H. armigera*, it is important to consider resistance management measures for Bt cotton in northwestern China.

Keywords Bt cotton · Susceptibility · Farming type · Resistance management · Seed mix refuge

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Key message

- Bt cotton has been cultivated for two decades without official authorization in northwestern China, and the susceptibility of *H. armigera* to Cry1Ac and Cry2Ab toxic protein has not been well characterized.
- Susceptibility of *H. armigera* to Cry1Ac and Cry2Ab is still high in northwestern China, and farming type does not influence susceptibility to these Bt toxins.
- IRM (Insect Resistance Management) for Bt cotton should be implemented, and actions to limit dominant resistant individuals spreading from northern China should be taken into consideration.

Introduction

Transgenic crops have been commercially adopted for over 20 years worldwide, and the total area planted annually has reached more than 185 million hectares in 26 countries

(James 2016). Economic losses caused by pest Lepidoptera in crops have been greatly reduced by the adoption of Bt crops with concomitant reduction in the use of broad-spectrum insecticide and environmental pollution in cotton production areas (Han et al. 2016; Downes et al. 2010, 2017; Wan et al. 2017; Dively et al. 2018; Wilson et al. 2018). As in the case of insecticides, evolution of resistance to Bt crops threatens the sustainability of this strategy for pest management (Tabashnik et al. 2008, 2013; Huang et al. 2010; Ives et al. 2017). Of the 13 major target pests for Bt crops, field-evolved resistance has been reported in five species at least (Tabashnik et al. 2013; Van Rensburg 2007; Gassmann et al. 2011; Grimi et al. 2018).

The cotton bollworm, *Helicoverpa armigera* (Hübner), is a highly polyphagous cosmopolitan pest on various crops, particularly on cotton (Cunningham and Zalucki 2014; Downes et al. 2017). In China, Bt cotton expressing a single toxin, Cry1Ac protein, has been widely used to curb this pest in cotton-growing areas (Wu 2007; Zhang et al. 2011; Chen et al. 2017). This situation differs from that in USA, Australia and India, where cotton containing pyramids of two or more Bt genes (mostly expressing Cry1Ac and Cry2Ab) is used (Chen et al. 2017; Liu et al. 2017). The susceptibility of *H. armigera* to Bt cotton has dramatically decreased over time in northern China (Wu 2007; Li et al. 2007; Liu et al. 2008, 2010; Zhang et al. 2018). Of concern are the presence of diverse resistance alleles and the apparent increasing percentage of dominant resistance in field-selected populations (Zhang et al. 2012; Jin et al. 2013). The percentage of resistant insects with non-recessive resistance reportedly has increased in northern China, from 37% of the 0.93% resistant insects in 2010 to 84% of the 5.5% resistant insects in 2013 (Jin et al. 2015). These trends pose a significant challenge to IRM in China, especially in northwestern China, where Bt cotton has been cultivated intensively for almost two decades although not officially authorized, and now represents more than 70% of the cotton production in China and 8–10% of worldwide cotton production (Wu and Guo 2005; Li et al. 2014; Wang et al. 2018). Even so, the status of *H. armigera* susceptibility to Cry1Ac Bt cotton has not been routinely monitored in this area of China.

Because of superior planting conditions for cotton cultivation, cotton production has greatly increased in Xinjiang Uygur Autonomous Region (called Xinjiang) and cotton makes up a much higher proportion of crop land than provinces in northern China (Fig. 2a; Wu and Guo 2005; Yang et al. 2017a). In contrast, the adoption of Bt cotton in Xinjiang is much lower than other provinces in China (Qiao 2015), partly because its planting does not have official approval. In Xinjiang, Bt cotton was first planted in 1997 and cotton covered about 34% of the total farmland area in 2014 (Li et al. 2013), with the percentage of Bt cotton ca 53% of total cotton in 2012. In comparison, the percentage of Bt

cotton planted in six provinces of northern China increased from 11% in 1998 to 50% in 2000, 91% in 2004 and nearly 100% of total cotton by 2014 (Wu et al. 2008; Zhang et al. 2011; Qiao 2015) but comprised less than 10% of total farmland in this cotton area. This suggests that the potential natural refuge for *H. armigera* in this cotton production region is much higher than that in Xinjiang.

In a desert region such as Xinjiang, the nature of topography and availability of water restricts cotton to a series of relatively small, isolated areas where irrigation is available, effectively islands in a sea of desert and potentially “local” *H. armigera* populations (Lu and Baker 2013; Lu et al. 2013; Gu et al. 2018). This may restrict the movement and mating of *H. armigera* among cotton production areas, thus increasing the selection pressure and speeding the evolution of resistance (Ives et al. 2017). Moreover, the pattern of planting systems (i.e., the coexistence of broad-acre and small-holder farming) in Xinjiang might influence the susceptibility of *H. armigera* due to differences in the amount of natural refuges between the two types of farming (Li et al. 2014). Finally, much of the cotton seed comes from unknown sources (Huang et al. 2014), and the quality of Bt cotton seed being sold is not clear, particularly its toxin protein expression. Thus, it is important to evaluate the susceptibility of *H. armigera* to Bt toxin in Xinjiang, given the increasing percentage of non-recessive resistance in northern China (Jin et al. 2015). In addition, pyramided Bt cotton expressing both Cry1Ac and Cry2Ab is expected to be commercialized throughout China, including northwestern China (Jin et al. 2015). Therefore, it is necessary to establish and monitor the susceptibility of *H. armigera* to both Cry2Ab and Cry1Ac in northwestern China.

To aid IRM planning for northwestern China, the susceptibility of *H. armigera* to Cry1Ac and Cry2Ab was assessed in nine populations across Xinjiang in 2014–2015. We (1) assess the susceptibility of *H. armigera* to Cry1Ac after two decades of intensive Bt cotton planting without any mandated IRM policy; (2) measure baseline susceptibility to Cry2Ab in *H. armigera* in the absence of Cry2Ab release in this area; and (3) evaluate the effect of farming system (broad-acre vs. small-holder farming) on the susceptibility *H. armigera* to Bt toxins. This work provides the first baseline data for cotton bollworm susceptibility to Cry2Ab and updates susceptibility data for Cry1Ac in a situation representing increasing use of Bt crops in this area.

Materials and methods

Collection of insect populations

Larvae (> 3 instar) of *H. armigera* were collected in fields of different crops locally (mainly tomato and corn, see

Table 1 Concentration responses to Cry1Ac protein of *H. armigera* larvae as measured by LC₅₀ and IC₅₀ for nine populations established from field collections from crops adjacent to cotton in Xinjiang in 2014–2015 (see Fig. 1 for details)

| Year | Area | Population | N | LC ₅₀ (µg ml ⁻¹) | 95%CI | Slope | IC ₅₀ (ug ml ⁻¹) | 95%CI | Slope | Host |
|------------------|--------------------|------------|--------------------|---|-------------|-------|---|-------------|-------|-------------|
| 2014 | Small-holder farms | Shache | 400 | 16.94 | 10.39–18.87 | 0.1 | 0.045 | 0.006–0.289 | 3.6 | Corn |
| | | Awat | 300 | 6.14 | 4.49–11.04 | 0.3 | 0.093 | 0.000–0.279 | 6.9 | Corn |
| | | Xinhe | 300 | 6.71 | 5.35–10.70 | 0.3 | 0.013 | 0.009–0.098 | 7.2 | Corn |
| | | Korla | 300 | 5.49* | 4.59–7.37 | 0.4 | 0.018 | 0.006–0.068 | 5.2 | Tomato |
| | | Shawan | 350 | 14.80 | 11.19–16.17 | 0.1 | 0.098 | 0.00–1.076 | 8.2 | Tomato |
| | Broad-acre farms | Alar | 352 | 10.51 | 7.22–12.42 | 0.3 | 0.121 | 0.007–0.664 | 4.4 | Corn |
| | | B29 | 300 | 6.72 | 5.21–11.08 | 0.3 | 0.068 | 0.000–0.327 | 7.1 | Tomato |
| | | B86 | 250 | 11.94 | 10.27–19.26 | 0.1 | 0.474* | 0.372–0.650 | 3.1 | Tomato |
| | | B124 | 250 | 8.62 | 6.85–14.15 | 0.3 | 0.430 | 0.105–1.125 | 1.8 | Tomato |
| | | 2015 | Small-holder farms | Shache | 250 | 8.08 | 5.71–12.60 | 0.2 | 0.231 | 0.124–0.356 |
| Awat | 300 | | | 6.67 | 3.28–15.21 | 0.1 | 0.679 | 0.308–2.122 | 1.3 | Corn |
| Xinhe | 300 | | | 5.39 | 3.14–12.52 | 0.2 | 0.439 | 0.261–1.091 | 2.6 | Corn |
| Korla | 300 | | | 3.16 | 1.45–9.82 | 0.4 | 0.115 | 0.000–1.984 | 5.8 | Tomato |
| Shawan | 260 | | | 5.96 | 3.83–18.10 | 0.2 | 0.182 | 0.102–0.317 | 5.2 | Tomato |
| Broad-acre farms | Alar | | 350 | 7.15 | 6.41–15.98 | 0.7 | 0.741 | 0.143–2.947 | 1.1 | Corn |
| | B29 | | 300 | 6.46 | 5.55–14.50 | 0.5 | 0.376 | 0.329–0.426 | 4.6 | Tomato |
| | B86 | | 200 | 9.08 | 5.74–17.84 | 1.8 | 0.172 | 0.086–0.515 | 7.2 | Tomato |
| | B124 | | 280 | 4.28 | 2.53–12.23 | 0.4 | 0.219 | 0.109–0.406 | 6.6 | Wheat |

LC₅₀ and IC₅₀ values designated by * are significantly different from each other based on non-overlap of 95% fiducial limits. LC₅₀—the toxin concentration (ug ml⁻¹) killing 50% of larvae (dead larvae), and IC₅₀—the toxin concentration (ug ml⁻¹) resulting in growth inhibition of 50% of larvae (dead to second-instar larvae)

Table 2 Concentration responses to Cry2Ab protein of *H. armigera* larvae as measured by LC₅₀ and IC₅₀ for nine populations established from field collections from crops adjacent to cotton in Xinjiang in 2014–2015 (see Fig. 1 for details)

| Year | Area | Population | N | LC ₅₀ (µg ml ⁻¹) | 95% CI | Slope | IC ₅₀ (µg ml ⁻¹) | 95% CI | Slope | Host |
|------------------|--------------------|------------|--------------------|---|-------------|-------|---|-----------|-------|-----------|
| 2014 | Small-holder farms | Shache | 400 | 8.42 | 7.16–12.22 | 0.2 | 0.67 | 0.33–1.96 | 0.7 | Corn |
| | | Awat | 300 | 6.47 | 4.27–9.54 | 0.3 | 1.04 | 0.15–3.88 | 0.8 | Corn |
| | | Xinhe | 300 | 4.61 | 3.41–7.46 | 0.3 | 0.41 | 2.02–4.44 | 1.3 | Corn |
| | | Korla | 300 | 5.88 | 4.03–13.98 | 0.3 | 0.75 | 0.19–3.70 | 1.2 | Tomato |
| | | Shawan | 350 | 3.43 | 2.05–7.95 | 0.3 | 0.16 | 0.06–1.24 | 4.3 | Tomato |
| | Broad-acre farms | Alar | 352 | 6.24 | 3.69–9.46 | 0.2 | 0.60 | 0.38–0.88 | 1.1 | Corn |
| | | B29 | 300 | 6.21 | 4.33–15.38 | 0.3 | 1.15 | 0.74–1.76 | 1.1 | Tomato |
| | | B86 | 250 | 8.41 | 6.69–14.39 | 0.3 | 1.45 | 0.80–2.73 | 1.0 | Tomato |
| | | B124 | 250 | 4.85 | 2.78–9.23 | 0.3 | 0.47 | 0.22–0.97 | 0.8 | Tomato |
| | | 2015 | Small-holder farms | Shache | 250 | 8.61 | 6.27–12.91 | 0.2 | 0.51 | 0.39–1.32 |
| Awat | 300 | | | 19.05* | 17.21–22.53 | 0.1 | 2.07 | 1.13–3.18 | 6.9 | Corn |
| Xinhe | 300 | | | 7.41 | 4.47–8.96 | 0.2 | 3.06 | 2.02–4.44 | 7.2 | Corn |
| Korla | 300 | | | 6.74 | 4.50–9.87 | 0.2 | 0.94 | 0.00–4.30 | 5.2 | Tomato |
| Shawan | 260 | | | 12.32 | 7.95–14.15 | 0.1 | 0.88 | 0.00–1.71 | 8.2 | Tomato |
| Broad-acre farms | Alar | | 350 | 6.19 | 5.13–11.08 | 0.3 | 3.81* | 3.25–4.53 | 4.4 | Corn |
| | B29 | | 300 | 7.15 | 4.18–9.21 | 0.2 | 1.82 | 0.80–3.37 | 7.1 | Tomato |
| | B86 | | 200 | 7.17 | 6.0–9.44 | 0.3 | 3.39 | 2.38–4.88 | 3.1 | Tomato |
| | B124 | | 280 | 7.46 | 5.24–10.27 | 0.3 | 1.98 | 1.14–3.10 | 1.8 | Wheat |

LC₅₀ and IC₅₀ values designated by * are significantly different from each other based on non-overlap of 95% fiducial limits. LC₅₀—the toxin concentration (ug ml⁻¹) killing 50% of larvae (dead larvae), and IC₅₀—the toxin concentration (ug ml⁻¹) resulting in growth inhibition of 50% of larvae (dead to second-instar larvae)

Tables 1 and 2 for details) adjacent to cotton fields during the cotton-growing season in 2014 and 2015. The sampled sites were selected on the basis of distribution and cropping characteristics (broad-acre vs. small-holder farming) in Xinjiang (Fig. 1). In 2014, fields in each region were chosen in cropping landscapes dominated by cotton, and these fields were revisited in 2015. At least 250 larvae (400 in some cases) were collected from each sampled field or an immediately adjacent field if larval numbers were low (less than 50). Larvae from each sampled crop site were transferred onto fresh artificial diet upon arrival in the laboratory and reared using a standard protocol (Zhang et al. 2011). Consequently, nine populations representing each sampling site were established. For each population, the male and female pupae were separated after larvae pupated. When most of the adults had emerged, 40–50 adults were put together (sex ratio 1:1) for mating in a container covered with a piece of gauze. There were 10–20 containers for each sampled site. Eggs (300–500) were collected from each container and put together based on sampled sites. The neonate larvae were used for bioassays.

Bt proteins

The Cry1Ac and Cry2Ab pro-toxin powders used in bioassays were supplied by Beijing General Pest Biotech Research Co. Ltd. The Bt proteins were separately incorporated into artificial diet for the bioassays (Sims et al. 1996). We chose the test concentrations in our study based on previous similar studies (Wu et al. 1999; Bird and Akhurst 2007; Anilkumar et al. 2008; Brévault et al. 2009). Stock suspensions of Cry1Ac and Cry2Ab were diluted with distilled water to produce six diet concentrations of Cry1Ac (0.005, 0.05, 0.5, 2.5, 5 and 25 $\mu\text{g ml}^{-1}$) and Cry2Ab (0.05, 0.5, 2.5, 5, 10 and 25 $\mu\text{g ml}^{-1}$). Distilled water was used as the control in experiments with both Cry1Ac and Cry2Ab.

Bioassays

Two ml of Bt-containing diet was poured into each well of 24-well insect assay trays. Newly hatched (< 24 h) unfed and active larvae were transferred onto the diet with a fine brush (1 larva/well). Trays were covered with plastic ventilated covers and kept in an incubator at $27 \pm 1^\circ\text{C}$. Twenty-four larvae from each sampled population were

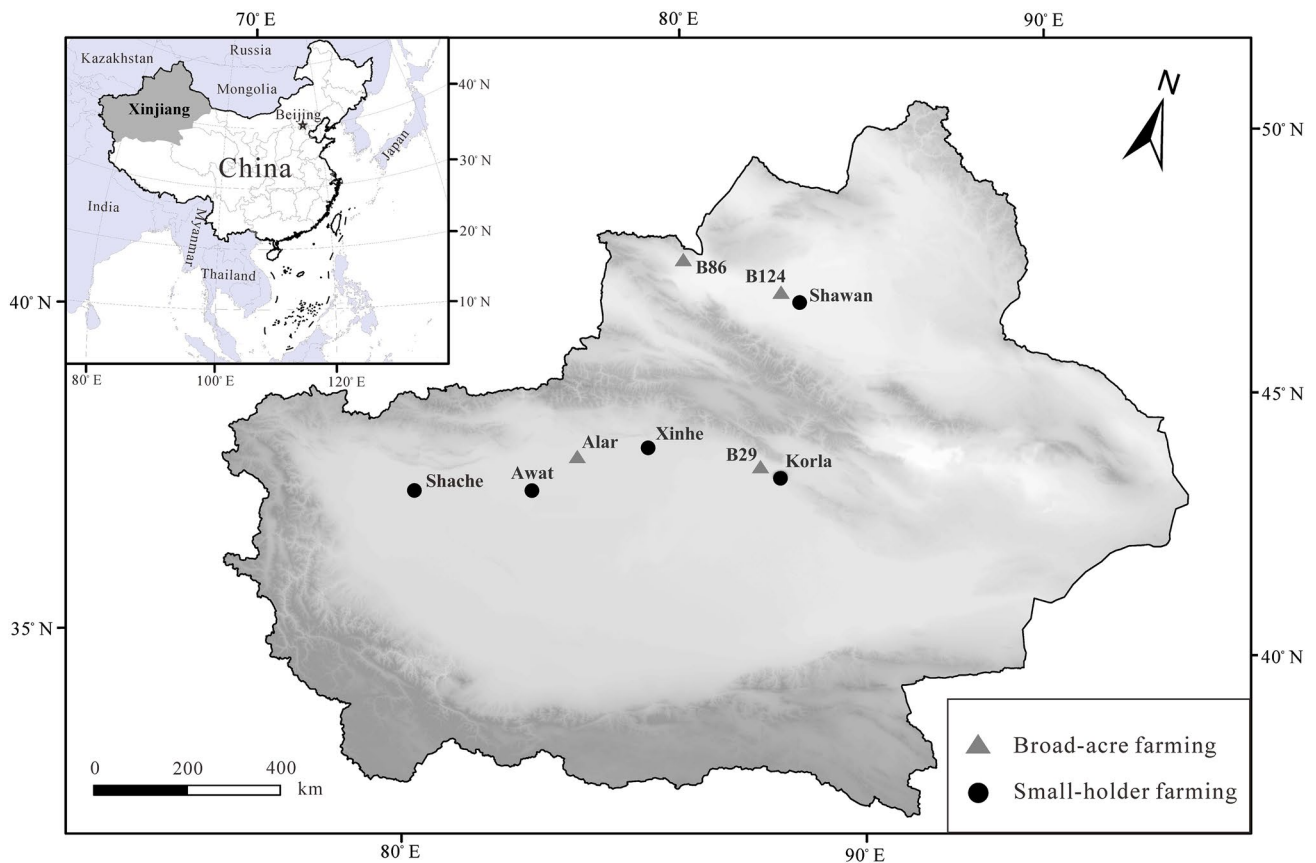


Fig. 1 Sampling area where *H. armigera* larvae were collected in Xinjiang in 2014–2015 (see Table 1 for details)

used for each concentration and for the untreated control, and the assay was repeated five times with each population of *H. armigera*.

Based on previous studies on baseline susceptibility of lepidopteran pests to Bt proteins, two parameters were measured in bioassays at 7 days: mortality, defined as individuals showing no reaction to gentle probing (Sims et al. 1996; Avilla et al. 2005; Wu et al. 1999) and larval growth inhibition based on larval weights (Wu et al. 1999; Bird and Akhurst 2007).

Data analysis

Probit analysis of the data was carried out using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) to compute 50% lethal concentrations (LC₅₀) and 50% inhibitory concentrations (IC₅₀). Two populations were considered significantly different in their response if their 95% confidence limits did not overlap (Jalali et al. 2004; Liao et al. 2002; Chandrashekar et al. 2005).

Independent t tests were used to evaluate the effect of farming systems (broad-acre and small-holder farming) and date (2014 and 2015). Pearson correlation analysis was used to estimate the correlation between responses to Cry1Ac and Cry2Ab (Brévault et al. 2009).

Results

Susceptibility of *H. armigera* to Cry1Ac

The LC₅₀ and IC₅₀ values varied between years as well as among populations. The responses among the nine *H. armigera* populations to Cry1Ac in 2014 ranged from 5.49 to 16.94 µg ml⁻¹ for LC₅₀ and for IC₅₀ from 0.013 to 0.474 µg ml⁻¹. In 2015, these values ranged from 3.16 to 9.08 µg ml⁻¹ for LC₅₀ and 0.115 to 0.741 µg ml⁻¹ for IC₅₀. The populations in 2014 and 2015 were compared within years based on non-overlap of 95% fiducial limits (Table 1). There were no significant differences among populations in 2014 except for Korla (for LC₅₀) and B86 (for IC₅₀) (Table 1). In 2015, there were no significant

differences among populations for LC₅₀, as well as IC₅₀ (Table 1).

Baseline susceptibility of *H. armigera* to Cry2Ab

Similar to Cry1Ac, there was variability among populations in the susceptibility of *H. armigera* to Cry2Ab. The responses of nine *H. armigera* populations to Cry2Ab in 2014 ranged from 3.43 to 8.42 µg ml⁻¹ for LC₅₀ and 0.16 to 1.45 µg ml⁻¹ for IC₅₀. The 95% fiducial limits of the data indicated that there were no significant differences in LC₅₀ and IC₅₀ among different populations (Table 2). In 2015, the responses of nine *H. armigera* populations to Cry2Ab ranged from 6.19 to 19.05 µg ml⁻¹ for LC₅₀ and from 0.51 to 3.81 µg ml⁻¹ for IC₅₀. The 95% fiducial limits of the data indicated that there were no significant differences in LC₅₀ among the different populations except for Awat, and no significant differences in IC₅₀ except for Alar (Table 2).

Susceptibility of *H. armigera* to Cry1Ac versus Cry2Ab

Pearson correlation analysis was used to estimate the correlation between Cry1Ac and Cry2Ab responses. Across the nine field populations, there were no significant correlations between Cry1Ac and Cry2Ab susceptibility for any of the two measurements in 2014 (LC₅₀: *r* = 0.252, *p* = 0.514; IC₅₀: *r* = 0.335, *p* = 0.378) nor in 2015 (LC₅₀: *r* = 0.097, *p* = 0.805; IC₅₀: *r* = 0.558, *p* = 0.119).

The effect of farming systems and date

For both Cry1Ac and Cry2Ab farming type (small-holder and broad-acre farming) did not affect LC₅₀ and IC₅₀. For years (2014 and 2015), LC₅₀ in 2015 was significantly lower than that in 2014, but there was no difference for IC₅₀ for Cry1Ac. There was no significant difference in LC₅₀ for Cry2Ab susceptibility between years (Table 3).

Discussion

Our work provides the first baseline data on cotton bollworm susceptibility to Cry2Ab and updated information on susceptibility to Cry1Ac following the increasing use of Bt crops in

Table 3 Independent *t* tests on resistance level between farm types and years, respectively

| | Cry1Ac | | Cry2Ab | |
|------------------|---|-----------------------------------|---|-----------------------------------|
| | Farm type (broad-acre vs. small-holder) | Year (2014 vs. 2015) | Farm type (broad-acre vs. small-holder) | Year (2014 vs. 2015) |
| LC ₅₀ | <i>t</i> = 0.092, <i>p</i> > 0.05 | <i>t</i> = 2.358, <i>p</i> < 0.05 | <i>t</i> = 0.97, <i>p</i> > 0.05 | <i>t</i> = 2.065, <i>p</i> > 0.05 |
| IC ₅₀ | <i>t</i> = 1.295, <i>p</i> > 0.05 | <i>t</i> = 2.082, <i>p</i> > 0.05 | <i>t</i> = 1.596, <i>p</i> > 0.05 | <i>t</i> = 3.168, <i>p</i> < 0.05 |

northwestern China. The baseline susceptibility to Cry2Ab in Xinjiang was similar to that in other parts of the world (“Susceptibility baseline of Cry2Ab in northwestern China” section), and the susceptibility to Cry1Ac was higher than in northern China (“Level of susceptibility of *H. armigera* field populations to Cry1Ac Bt cotton” section).

Level of susceptibility of *H. armigera* field populations to Cry1Ac Bt cotton

In our study, the lack of a susceptible strain makes it difficult to assess the evolution of resistance of *H. armigera* to Cry1Ac since the release of Bt cotton in Xinjiang. Our data on susceptibility of cotton bollworm to Cry1Ac can be compared with those from other studies that included a susceptible strain. The two populations in northwestern China described by Zhang et al. (2011) were collected in Shache and Shawan in Xinjiang, which overlap with our sampling areas (Fig. 1). The susceptibility of these two populations was at the same level as that of a susceptible laboratory strain of *H. armigera* (Zhang et al. 2011). We take the Shache and Shawan populations from Xinjiang to be indicative of a susceptible strain in our study. The median LC_{50} of Cry1Ac for 13 populations from northern China measured by Zhang et al. (2011) was 2.8 times higher for activated toxin and 3.0 times higher for protoxin than for Shache and Shawan populations in Xinjiang. More recently Zhang et al. (2018) found that the susceptibility of *H. armigera* to Cry1Ac in Xinjiang (as measured by toxin concentration causing 50% inhibition of larval development to the third instar) is higher than that from Huanghe River Valley and Yangtze River Valley cotton areas. This is indirect evidence to suggest that *H. armigera* in Xinjiang remains relatively susceptible to Cry1Ac but we note that the inference needs to be treated with caution as the two studies used different methods.

The stability of resistance allele frequencies also supports a conclusion of continued Cry1Ac susceptibility of *H. armigera* in Xinjiang. Li et al. (2010) reported that from 2005 to 2009, the resistance allele frequency in Korla fluctuated between 0.000 and 0.004 and from 0.0000 to 0.0008 in individuals collected from Shache. In 2010–2011, the frequencies of Cry1Ac resistance in *H. armigera* populations were $< 10^{-3}$ in both Shihezi and Shache (Wang et al. 2012). These results indicate that resistance allele frequencies in populations of *H. armigera* in northwestern China were at low levels, which is consistent with the bioassay results in our study.

Possible reasons for continued *H. armigera* susceptibility to Cry1Ac Bt cotton

Xinjiang has a lower level of alternate host refuge than northern China (Fig. 2b) and the lack of a clear policy on

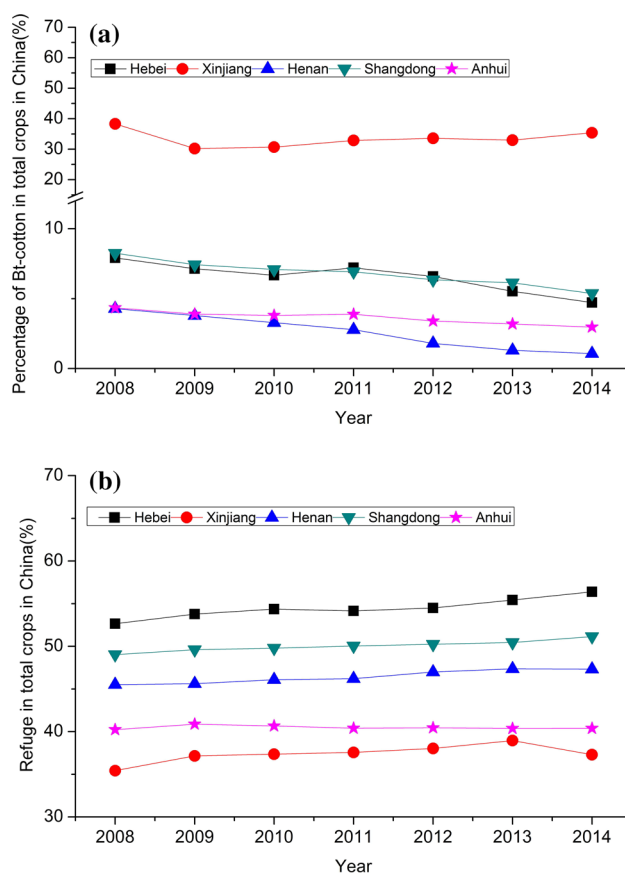


Fig. 2 Cotton planted as percentage in total crops in five provinces of China from (China Rural Statistical Yearbook 2008–2014) (a), and refuge area as a percentage in total crops in five provinces of China according to Huang’ method (2010) (b)

Bt cotton there might, theoretically, lead to rapid resistance evolution. However, the susceptibility of *H. armigera* to Cry1Ac remains at levels similar to that in northern China.

One possibility is that seed mix refuges might delay or mitigate resistance development to Bt cotton as on other Bt crops (Carroll et al. 2012, 2013; Carrière et al. 2016; Wan et al. 2017). The percentage of non-Bt cotton seed in seed lots was 10–30% in local markets because of poor market management in Xinjiang (Lu, unpublished data). The non-Bt cotton mixed in Bt cotton fields might serve as refuge and delay resistance evolution if larvae of *H. armigera* have low mobility between Bt cotton and non-Bt cotton and high inherent susceptibility to Bt toxins. The effectiveness of seed mixtures to counter the evolution of resistance is controversial (Brevault et al. 2015; Carrière et al. 2016; Yang et al. 2017b). However, the use of seed mixtures successfully mitigated against the development of resistance at least experimentally, for example the pink bollworm (*Pectinophora gossypiella*) in China (Wan et al. 2017).

Susceptibility baseline of Cry2Ab in northwestern China

The baseline susceptibility of Cry2Ab was 3.43–19.05 $\mu\text{g ml}^{-1}$ (LC_{50}) and 0.16–3.81 $\mu\text{g ml}^{-1}$ (IC_{50}) over two consecutive years in our study region where Bt cotton expresses single Cry1Ac toxin. Similar variability in the response of *H. armigera* populations to Cry2Ab toxin has been reported in other areas: 6–28.6 $\mu\text{g ml}^{-1}$ (India) and 5.12–50.71 $\mu\text{g ml}^{-1}$ (West Africa) for LC_{50} ; 0.14–0.60 $\mu\text{g ml}^{-1}$ (Australia), 0.31–2.3 $\mu\text{g ml}^{-1}$ (India) and 0.22–8.72 $\mu\text{g ml}^{-1}$ (West Africa) for IC_{50} (Bird and Akhurst 2007; Brévault et al. 2009; Anilkumar et al. 2008).

Zhang et al. (2011) found that the median LC_{50} of Cry2Ab for 15 populations indicated no significant difference between northern (13 populations) and northwestern China (two populations); the Shache and Shawan populations collected in 2010 from Xinjiang. Moreover, the susceptibility of *H. armigera* populations to Cry2Ab in Shache and Shawan was not significantly different from that of the susceptible laboratory strains of this species. In our study, the LC_{50} values of Cry2Ab for nine populations throughout Xinjiang, including Shache and Shawan populations, were not significantly different from each other except for Awat in 2015 (Table 2). These results indicate that the susceptibility of *H. armigera* to Cry2Ab in northwestern China is still higher in the Bt cotton areas as expected.

Lack of cross-resistance between Cry2Ab and Cry1Ac

The high susceptibility to Cry2Ab across populations in northwestern China and the lack of correlation between Cry2Ab and Cry1Ac susceptibility indicate that any resistance to Cry1Ac that may be present has not conferred cross-resistance to Cry2Ab. Our results are consistent with many studies across a range of target pests that includes *H. armigera* and other *Helicoverpa* species, including studies of *H. armigera* in Australia and western Africa (Brévault et al. 2009), but not with the cross-resistance found between Cry1Ac and Cry2Ab in one study from northern China (Gao et al. 2009). Our results indicate that Bt cotton containing Cry2Ab could be very useful against populations with resistance to Cry1Ac, at least in Xinjiang.

Rational IRM to Bt cotton in northwestern China

Even though *H. armigera* still exhibits a high level of susceptibility to Bt cotton in Xinjiang, a policy on Bt cotton adoption and IRM should be developed to manage seed markets in this region. Actions need to be considered to restrict

dominant resistant individuals increasing locally or moving from northern China to northwestern China.

Cotton only makes up 4–10% of the sown area in northern China of which 98% is Bt cotton without a mandatory refuge (Fig. 2 and Huang et al. 2010). Although there are large areas of natural refuge including corn, soybean and vegetables in northern China (Ye et al. 2015), resistance appears to be on the rise, particularly the frequency of individuals with dominant resistance (Jin et al. 2015). The significant concern is how long can one avoid mandatory refuges in northwestern China and a policy for their implementation? Mandatory refuges are considered one of the key contributors to successful IRM in Australia (Downes et al. 2017; Wilson et al. 2018). An active IRM program should be developed for Xinjiang as far less natural refuge is available, particularly in broad-acre farming districts (Lu et al. 2013). The optimization of multiple refuge hosts temporally and spatially across landscapes is crucial for the production of susceptible moths that are likely to mate with resistant moths (Baker et al. 2008; Baker and Tann 2013; Lu et al. 2013; Li et al. 2017), thus improving the efficacy of IRM. Moreover, after a careful risk assessment, pyramided Bt cotton should be released to provide an additional tool to combat potential Bt resistance issues in northern China (Chen et al. 2017; Liu et al. 2017).

Author contribution statement

ZZL and MPZ designed research, PPW, JHM and DPX conducted bioassays, JL, HQW collected samples, ZMX and GPH analyzed data, and MPZ and ZZL wrote this manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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