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# Early detection of field-evolved resistance to Bt cotton in China: Cotton bollworm and pink bollworm

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#### ABSTRACT

Transgenic crops producing *Bacillus thuringiensis* (Bt) toxins kill some major insect pests, but pests can evolve resistance and thereby reduce the effectiveness of such Bt crops. The main approach for slowing pest adaptation to Bt crops uses non-Bt host plants as "refuges" to increase survival of susceptible pests. To delay evolution of pest resistance to cotton producing Bt toxin Cry1Ac, several countries have required refuges of non-Bt cotton, while farmers in China have relied on "natural" refuges of non-Bt host plants other than cotton. This strategy is designed for cotton bollworm (*Helicoverpa armigera*), which attacks many crops and is the primary target of Bt cotton in China, but it does not apply to pink bollworm (*Pectinophora gossypiella*), which feeds almost entirely on cotton in China. Here we review evidence of field-evolved resistance to Cry1Ac by cotton bollworm in northern China and by pink bollworm in the Yangtze River Valley of China. For both pests, results of laboratory diet bioassays reveal significantly decreased susceptibility of field populations to Cry1Ac, yet field control failures of Bt cotton have not been reported. The early detection of resistance summarized here may spur countermeasures such as planting Bt cotton that produces two or more distinct toxins, increased planting of non-Bt cotton, and integration of other management tactics together with Bt cotton.

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#### 1. Introduction

Transgenic crops that produce insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) kill some key pests and can help to reduce reliance on insecticide sprays (Sanahuja et al., 2011). Such Bt crops were commercialized in 1996 and covered more than 66 million hectares worldwide in 2011 (James, 2011). The primary threat to the continued success of Bt crops is evolution of resistance by pests (Gould, 1998; Tabashnik, 1994). Field-

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evolved (=field-selected) resistance entails a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field (Tabashnik et al., 2008, 2009a). Although many target pest populations remain susceptible, some degree of field-evolved resistance to Bt crops has been reported in at least nine species of target pests (Alcantara et al., 2011; Bagla, 2010; Carriere et al., 2010; Dhurua and Gujar, 2011; Downes et al., 2010b; Gassmann et al., 2011; Huang et al., 2011; Storer et al., 2010; Tabashnik et al., 2008, 2009b; Van Rensburg, 2007; Wan et al., 2012; Zhang et al., 2011). Field-evolved resistance to Bt toxins has caused field control failures in some, but not all cases.

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Here we focus on early detection of field-evolved resistance to Bt cotton in China in two major pests, cotton bollworm (Helicoverpa armigera) and pink bollworm (Pectinophora gossypiella). Bt cotton varieties planted in China are from Monsanto and the GK series developed in China (e.g., GK19 and GK12). The Monsanto varieties produce Cry1Ac and the GK varieties make a chimeric Bt toxin similar to Cry1Ac with amino acids 446-608 encoded by the cry1Ac gene and amino acids 1-445 encoded by the closely related cry1Ab gene (Guo, 1995). The percentage of China's Bt cotton accounted for by GK varieties was 5% in 1998, 50% in 2003, 70% in 2005 and 93% in 2009 (Yu and Fan, 2010). Unlike the situation in the United States and Australia, refuges of non-Bt cotton have not been required in China and Bt cotton producing a single toxin (Cry1Ac) has not been replaced by twotoxin cotton that produces both Cry1Ac and Cry2Ab (Downes et al., 2010a; Tabashnik et al., 2009b; Wu and Guo, 2005; Wu et al., 2008: Wan et al., 2012: Zhang et al., 2011).

In China, Bt cotton was commercialized in 1997 and has been effective against its main target, the cotton bollworm (Wu, 2007; Wu et al., 2008). China has not required non-Bt cotton refuges based on the premise that abundant non-Bt host plants of cotton bollworm other than cotton provide sufficient "natural" refuges to delay resistance in this pest (Wu, 2007; Wu and Guo, 2005; Wu et al., 2002). However, Bt cotton was introduced in 2000 in the Yangtze River Valley of China (Chinese Ministry of Agriculture, 2010), where pink bollworm is a major pest. The "natural" refuge concept does not apply to pink bollworm because it feeds almost exclusively on cotton, raising the risk of resistance (Wu and Guo, 2005).

In addition, although inherent susceptibility to Cry1Ac is greater for pink bollworm than for cotton bollworm, the concentration of Cry1Ac in Bt cotton varies over time, allowing survival of susceptible larvae of both pests during some of the growing season in China (Wu and Guo, 2005; Wan et al., 2004, 2005). Thus, a high dose of Cry1Ac is not maintained against either pest in China, which further increases the risk of resistance (Gould, 1998; Tabashnik, 2008; Wu and Guo, 2005). Below we review data providing early detection of resistance in both pests. We conclude by considering the implications of this resistance and potential proactive countermeasures to limit negative consequences of the resistance.

#### 2. Cotton bollworm resistance to Cry1Ac

Early evidence of field-evolved resistance to Cry1Ac in populations of cotton bollworm from China comes from at least seven studies based on the following comparisons between field populations with different histories of exposure to Bt cotton: northern versus northwestern China (Zhang et al., 2011), Anci versus Xiajin in northern China (An et al., 2010; Li et al., 2007; Wu et al., 1999; Zhang et al., 2011), Korla versus Shache in northwestern China (Li et al., 2010), and changes over time in northern China at both Qiuxian (Liu et al., 2010) and Anyang (Yang et al., 2007; Zhang et al., 2011). Here we review the results of Zhang et al. (2011) showing decreased susceptibility to Cry1Ac in northern China, where Bt cotton has been planted intensively, compared with Shawan and Shache of northwestern China, where Bt cotton planting has been limited.

Most of China's cotton grows in the area sometimes called northern China, which includes the Yangtze River Valley and the Yellow River Valley (Wu et al., 2008; Lu et al., 2010). In six provinces of northern China considered together, the percentage of cotton planted to Bt cotton increased from 11% in 1998 to 50% in 2000 and 91% in 2004, with 100% Bt cotton in some provinces by 2004 (Wu et al., 2008). By contrast, Bt cotton has not been planted



**Fig. 1.** Cotton bollworm resistance monitoring sites during 2010 (Zhang et al., 2011). Northern China (provinces of Hebei, Henan, Hubei, Jiangsu, and Shandong): c = Anci, Ay = Anyang, Gy = Gaoyang, Hm = Huimin, Jy = Juye, Kf = Kaifeng, Np = Nanpi, Ny = Nanyang, Qj = Qianjiang, Qx = Qiuxian, Qz = Quzhou, Xj = Xiajin, Yc = Yancheng. Northwestern China (Xinjiang Uygur Autonomous Region): Sc = Shache, Sw = Shawan.

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