Effect of farm management practices in the Bt *toxin production by* Bt *cotton: evidence from farm fields in China*

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ORIGINAL PAPER

Effect of farm management practices in the *Bt* toxin production by *Bt* cotton: evidence from farm fields in China

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Abstract Based on farm field plot level survey data and laboratory test, we examine the determinants of the expression of *Bt* toxin in China's *Bt* cotton production. The results show that the expression of *Bt* toxin differs significantly among varieties. Even for the same variety the expression of *Bt* toxin also varies substantially among villages and among farmers in the same village. Econometric analyses show that after controlling for the effects of varieties and locations (or villages), farm management, particular applications of phosphate and potash fertilizers, and manure, has significant positive effects on *Bt* toxin expression in farmer's fields. In contrast to previous studies which showed that nitrogen fertilizer has a positive impact on expression of *Bt* toxin, this study shows that nitrogen fertilizer has no significant impact on expression of *Bt* toxin in farmer's fields. On the other hand, the expression of *Bt* toxin has a positive relationship with phosphate fertilizer, potash fertilizer and manure application.

Keywords Expression of *Bt* toxin · Fertilizer use · Farm management · *Bt* cotton · China

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Introduction

The development of genetically modified (GM) crops has been the most successful application of agricultural biotechnology research to date. As the rapid spread in the world, 17.3 million farmers in 28 countries planted 170 million ha GM crops in 2012 (James 2012). As the fastest adopted crop technology in recent history, the GM crops deliver sustainable and substantial, socioeconomic and environmental benefits. The successful application of GM crops not only has contributed to the total agricultural production, but also to the alleviation of poverty and hunger worldwide (FAO 2001).

One of the main commercialized varieties of GM crops, *Bacillus thuringiensis* (*Bt*) transgenic crops, derives the resistance from the insecticide expressed by the gene of the bacterium *Bt* that is inserted into the DNA of the host crops. Even though cotton and maize engineered with such genes were grown commercially for the first time in 1996, their use has spread very quickly all over the world. In 2012, the total planting area of *Bt* only and *Bt* with herbicide tolerance maize and cotton was near 70 million ha in the world (James 2012). James (2012) also predicted that *Bt* crops would be planted on more arable land and in more countries in the future.

High dose is one of the most important criteria of selecting *Bt* crop varieties. Benefits of *Bt* plants come from their efficiency in controlling target pests, which include chemical pesticide use saving and yield increase (Pray et al. 2001; Huang et al. 2002, 2003; Qaim 2003). Previous studies showed that there was a close correlation between *Bt* protein content and controlling efficacy (for example, Holt 1998; Adamczyk et al. 2001; Kranthi et al. 2005). And the efficiency of *Bt* cotton depends upon the expression of Cry genes through synthesis of insecticidal protein in *Bt* crops (Rao 2005; Gutierrez et al. 2006). Hence if the *Bt* toxin content is low, the efficiency of *Bt* crops in controlling pests is low.

High dose is also crucial in the high-dose/refuge insecticide resistance management strategy. *Bt* technology appears to be a two-edged sword. On the one hand, *Bt* crops have been proven successful worldwide. On the other hand, the worry about the potential vulnerability of *Bt* crops to resistance adaptation by pests has never stopped (Qiao et al. 2008). To delay the development of resistance in the pest population, a "high-dose/refuge" strategy has been widely used in the countries where *Bt* crops are planted (Gould 1998; Baute 2004; Qiao et al. 2010). In this strategy, high dose varieties, which can at least kill 95 % of the heterozygotes for *Bt* resistance is a crucial requirement (Gould 1998).

The expression of *Bt* toxin has been affected by many factors. Previous studies showed that these factors not only include the genetic background of cotton varieties, but also environmental conditions and other factors (Adamczyk and Sumerford 2001; Zhang et al. 2001; Mahon et al. 2002; Shen et al. 2010). For example, previous studies have shown that light, temperature, irrigation, rainfall and other factors have significant impact on the expression of *Bt* toxin in transgenic crops (Cui and Xia 1999; Xing et al. 2001). In addition, Sachs et al. (1998) found the expression of *Bt* plant was quite different for the same cotton varieties if grown in different locations, and they argued that the difference was caused by the different environmental conditions.

The other important factor affecting the expression of *Bt* toxin is the nutrition status of the plant. For example Zhou et al. (2000) showed that the amount of fertilizer applied had significant impact on the expression of *Bt* toxin, and efficiency of *Bt* cotton decreases significantly if fertilizer application was not enough (or the nutrition status of the plant was bad). Further, Yang et al. (2005) and Coviella et al. (2002) showed that lack of nitrogen fertilizer decreased the expression of *Bt* toxin. However, all these studies were conducted in laboratory or small-scalar experimental fields where most of the inputs (such as fertilizer, pesticide, irrigation) were firmly controlled for special scientific objectives, which is totally different from farmer's crop production in practice. Consequently, the impact of agricultural inputs on the expression of *Bt* toxin in laboratory and experimental fields is expected to differ from that in farm fields that are operated by farmers.

Unfortunately, to the best of our knowledge, there is no study on the impact of farm management on the expression of *Bt* toxin in farm fields. Several questions are raised. For example, how does *Bt* toxin expression differ among varieties in farm fields? Does *Bt* toxin expression of one variety vary among villages, and/or among farmers in the same village? How importance of farm management, particularly fertilizer applications and planting saved seeds (which is a quite common practice in China, is in determining *Bt* toxin expression in farmer's fields?

The overall goal of this study is to empirically answer the above questions. To achieve this goal, we select *Bt* cotton in North China Plain as study area. North China Plain is an interesting case because it is one of major cotton production bases and the largest *Bt* cotton production region in China. This region is also the first region where *Bt* cotton was released due to the severity of pest infestation (Huang et al. 2002). Moreover, all cotton farmers are small in term of land area with average farm size of 0.7 ha or less (Huang et al. 2003). In addition, farm management, particular fertilizer applications, also differs largely among farmers in the sample area.

Materials and methods

Data

The field study was conducted in three provinces in North China Plain in 2007. These three provinces are Shandong, Hebei, and Henan, which are the second, third and fourth largest cotton production provinces in China (National Bureau of Statistics of China 2008).[1] In each province, two counties were randomly selected from the list of major cotton production counties. In each county, we selected two villages where cotton was a major crop. And in each village, 20 cotton households were randomly sampled. Finally, all the cotton plots of sampled households were included. Since some farmers have more than one cotton plots, this study includes 813 cotton plots. Two types of data set are used in this study: data from household farm management survey and laboratory test. The household farm management survey data were collected from the sampled households. Our questionnaire includes several blocks. In addition to survey blocks enumerating the basic characteristics of households (for example each household's land and labor endowments, farm size), there was one section that collected information that forms the core of this paper's analysis. In this section, detailed information about yields and inputs, such as seed (whether the seed is saved, seed price, etc.), irrigation, chemical fertilizer use, manure application, are recorded for each cotton plot.

In designing field experiment with farmers, we took large efforts to control seed quality. In practices, farmers usually purchase seeds from local and small seed companies, private peddlers, as well as other farmers. Due to the differences in production and processing procedures, quality of seeds that farmers purchase from different sellers varies. And planting saved seed is quite common in China. On the other hand, previous studies had shown that seed quality is an important factor affecting the expression of *Bt* toxin (for example, Wen et al. 2007). To have a better understanding of the *Bt* toxin expression of same variety with same quality in different locations and among farmers in the same location, we provided each farmer with one of six major *Bt* cotton varieties with free of charge. These seeds were bought by the project team from large and well known seed companies. To ensure uniform of seed quality for the same variety, we purchased each of these six varieties one time from one company only.

After we distributed our seeds to farmers, each farmer was asked to randomly assign one of his/her cotton plots to plant this variety. At the same time, the other cotton plots were planted with seeds that farmers purchased themselves from local markets. To increase the accuracy of data collection, rather than using a recall method, we asked each farmer to record his/her major farm management activities by plot, particularly irrigation, chemical fertilizer application and manure application, and other input and output information during the entire cotton production reason. In addition, we had visited sample farmers five times to assist farmer's farm management record and conducted face–face interviews.

Data from the laboratory test were collected and analyzed as follows. First, in each plot of cotton field, we collected cotton leaves three times in June, July and August, respectively. Each time, we selected five cotton plants (four from the corners and one in the center of the plot) in each plot. And then four pieces of leaves from each plant were picked. Second, after being collected, all leaves were immediately put into refrigerators filled with ice and then were shipped to the Institute of Plant Protection (IPP) of Chinese Academy of Agricultural Sciences in Beijing. Finally, IPP conducted the laboratory analysis of expression of *Bt* toxin for each of farms' plots in the three periods (June, July and August).

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[1] Xinjiang Uyghur Autonomous Region is the largest cotton production region in China. However, because of the hot and dry climate, the cotton bollworm is not a serious problem in Xinjiang.

Model

To understand the expression of *Bt* toxin and its determinants, we adopt a multivariate function approach and estimate the model using plot level data collected. Our objective is to estimate the *net* impact of different factors on the expression of *Bt* toxin. To do so, we need to hold the effect of many factors, such as cotton variety and characteristics of plot, constant. To achieve this objective, we set up and estimate a simple analytical framework as follows:

$$Bt\_expression_i = a_i + b_i * nitrogen_i$$
$$+ c_i * phosphate_i + d_i * potash_i$$
$$+ e_i * manure_i + f_i * saved\_seed_i$$
$$+ g_i * others_i + h_i * village\_dummies$$
$$+ i_i * variety\_dummies_i + u_i$$

where, the dependent variable, *Bt_expression* is the average expression of *Bt* toxin (ng/g) during the entire growing season (early, middle and late). *Nitrogen* is the amount of active nitrogen fertilizer applied per ha; *Phosphate* is the amount of active phosphate fertilizer applied per ha; *Potash* is the amount of active potash fertilizer applied per ha. *Manure* is a dummy variable representing whether manure is applied in the plot, which equals to 1 if yes, 0 otherwise. If the plot was planted with saved seed, *saved_seed* equals to 1, 0 otherwise.

Others is a vector of variables which include the soil quality (*soil*), the number of days between sowing and the first time when leaves were picked up (*sow*), years of continuous cropping cotton in the past (*replant*), and times of irrigation (*irrigation*). *Soil* is denoted by a dummy variable, which equals to 1 if the soil quality is good, 0 otherwise. The quality of soil was reported by farmers based on their own experience. Since the survey was conducted in 12 villages, 11 village dummies (*village_dummies*) were created and included in the equation to control the impact of climate factors and other factors which were same in one village. To control the effects of difference *Bt* cotton varieties, variety dummy variables (*variety-dummies*) were added. Finally, *i* is the ith plot; *u* is error term.

However, as discussed above, there is a strong correlation between phosphate fertilizer and potash fertilizer due to the wide use of compound fertilizer.

The statistical test also shows that the correlation coefficient between them is as high as 0.82. Hence, if both of these two variables appear in the function, multi-collinearity problem occurs. To solve this problem, we consider three scenarios: model (1) includes the phosphate fertilizer application but does not include the potash fertilizer application, model (2) includes the potash fertilizer application but does not include the phosphate fertilizer application, and model (3) includes the sum of phosphate fertilizer and potash fertilizer.

Results

Production of *Bt* toxin in farm fields

Figure 1 shows that there is a clear relationship between the expression of *Bt* toxin and the stages of plant growth. In this study, the expression of *Bt* toxin is measured by the nanogram (or ng) *Bt* toxin expressed per gram (or g) of leaves. As shown in Fig. 1, the expression of *Bt* toxin decreases sharply from the early growing season (June) to middle (July) and late (August) growing seasons in all the three provinces. According to our study, the expression level of *Bt* toxin decreases by more than 50 % from early growing season to late growing season.

This finding is consistent with previous studies which show that expression of *Bt* toxin can vary as much as twofold from early to late growing seasons (Adamczyk et al. 2001; Adamczyk and Sumerford 2001; Greenplate et al. 2001). As a result, it seems to
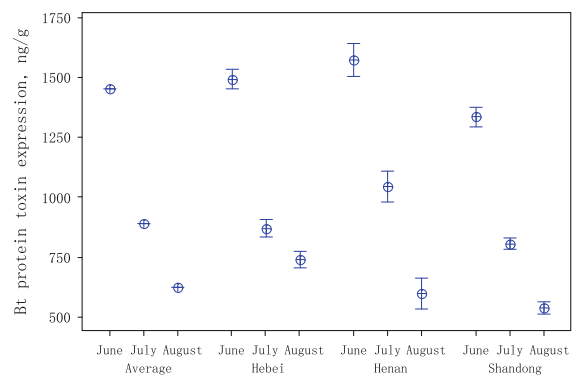

Fig. 1 Mean of *Bt* toxin (ng/g) expression with 95 confidence interval over different periods by province

Table 1 Expression of *Bt* toxin of different varieties

	Average	Early period (June)	Middle period (July)	Late period (August)
Varieties provided by this study				
HanZa154	1,089	1,480	1,102	684
ZhongMian45	1,521	1,914	1,714	935
LuMianYan21	918	1,436	793	527
LuMianYan15	878	1,265	845	524
JinMian26	911	1,290	765	677
NC20B	950	1,522	791	538
Varieties purchased by farmers				
FengKang1	1,109	1,673	867	786
ZhiShuMian	999	1,535	793	670
YinShuo116	1,088	1,639	761	865
LuMianYan28	922	1,401	861	504
Others	980	1,430	889	620
Total	986	1,451	888	620

be a common phenomenon that the efficacy of *Bt* cotton in controlling target pests is relatively high in early growing season, but significantly declines in late growing season (Greenplate 1999; Greenplate et al. 2000). During our field survey, we were also told that even though *Bt* cotton was efficient in controlling the second generation of cotton bollworm, the target pest of *Bt* cotton, at the early growing season (June), farmers still needed to spray some amount of chemical pesticides to control the third and fourth generations of cotton bollworm at the middle and late growing seasons.

As shown in Fig. 1, the expression of *Bt* toxin varies significantly among regions. For example, in the early growing season, the expression of *Bt* toxin in Henan province, on average, is significantly higher than that in Shandong province. Similar results can be found in middle and late growing seasons. As discussed in the "Introduction" section, the expression of *Bt* toxin is affected not only by seed quality and cotton varieties, but also environmental and climate conditions, farmer's field management, as well as other factors. In other words, the difference of expression of *Bt* toxin among regions found in Fig. 1 might be caused by one or more of these factors.

To see the likely impact of cotton variety, we first compare the average expression of *Bt* toxin among different varieties (Table 1). As discussed above, we

had purchased cotton seeds of six varieties from well-known large companies and distributed them to each household participated in this study. We believe that they are high quality seeds and expect that expression of *Bt* toxin of these varieties are higher than that of seeds that sampled farmers bought from local seed market. Since there are near 100 different cotton varieties were planted in our sample sites, we classify them into several groups before comparing the expression of *Bt* toxin. According to the number of observations of each variety, we classify these varieties that farmers purchased in the local seed markets into five groups: FengKang1, ZhiShuMian, YinShuo116, LuMianYan28, and others. Different from what we had expected, however, Table 1 shows that the six varieties of cotton seeds that we purchased from well-known large companies did not outperform those that farmers purchased from local markets (rows 1–6 vs. rows 7–11).

In order to control the impact of environmental and climate conditions, we then compare the expression of *Bt* toxin of each major variety in the same village. Since the seed quality of same variety might be different, in the following, we focus on the six varieties that we purchased to avoid the potential impact of seed quality within the same variety. As shown in Fig. 2, there is still significant difference of the expression of *Bt* toxin among households in each village. Since we are comparing the expression of *Bt* toxin of same cotton variety in the same village, we can control the impact of genetic characteristics of cotton varieties, climate and environmental conditions. In other words, the difference shown in Fig. 2 should come from farmer's farm management activities such as the fertilizer use, irrigation and others.

Farm management and *Bt* toxin expression

As mentioned earlier, during the field survey, we had collected the detailed information of fertilizer use in each cotton plot. Using these survey data, we can calculate the amount of active ingredients of nitrogen, phosphate, and potassium fertilizer applied in each plot, respectively. After conversion, we then can relate the expression of *Bt* toxin to the amount of active ingredients of different fertilizers use.

We first examine the relationship between the expression of *Bt* toxin and the amount of nitrogen fertilizer applied. According to the total amount of
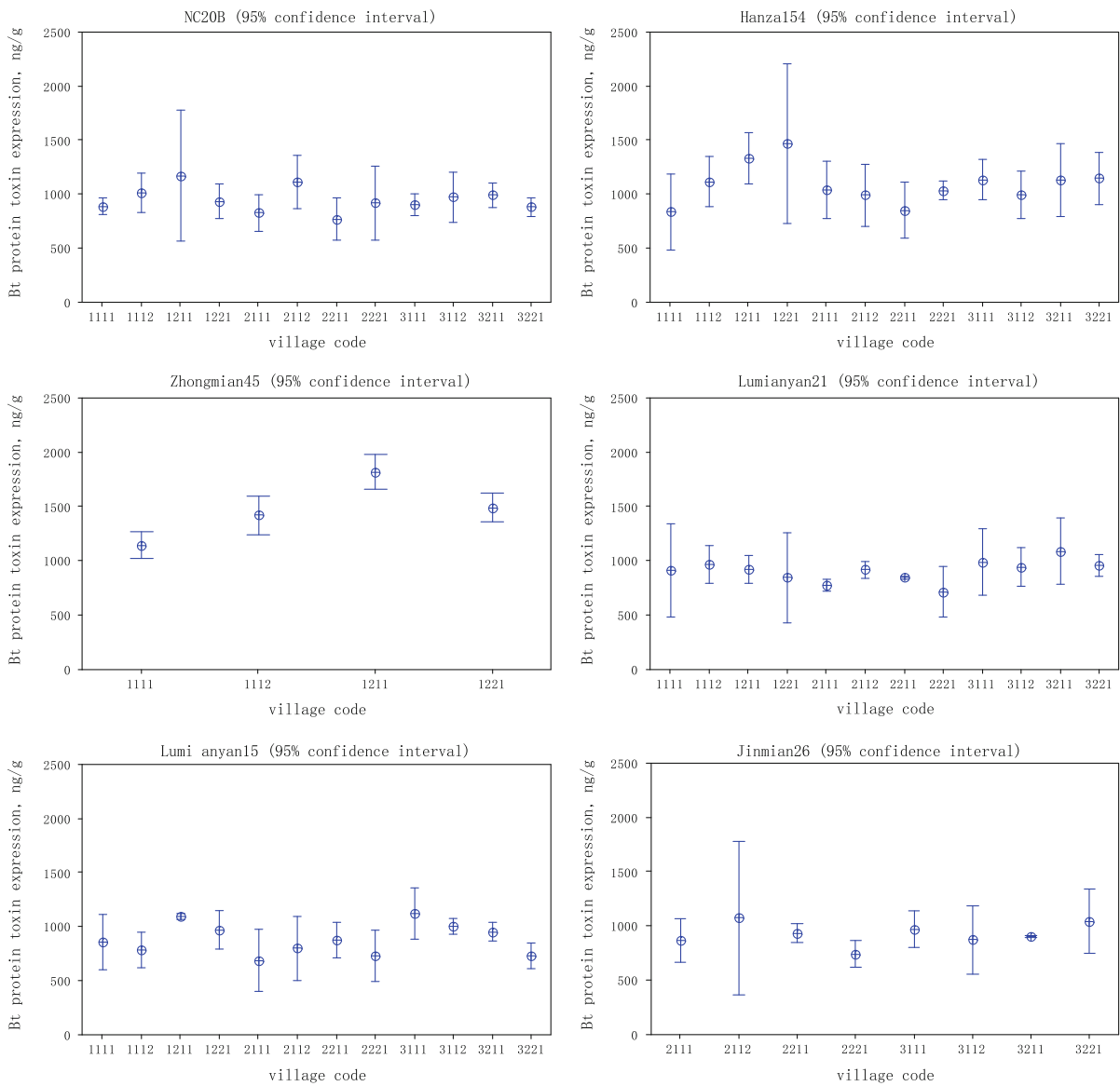
Fig. 2 Expression of *Bt* toxin (ng/g) by cotton variety and village

nitrogen fertilizer applied during the entire cotton growth season, we classify all the sample plots into three groups: low nitrogen fertilizer use group (total nitrogen fertilizer is up to 100 kg/ha), middle nitrogen fertilizer use group (total nitrogen fertilizer is greater than 100 kg/ha, but less than 200 kg/ha), and high nitrogen fertilizer use group (total nitrogen fertilizer is greater than 200 kg/ha). As shown in Table 2, the highest expression level of *Bt* toxin occurs in the middle nitrogen fertilizer use group, which is 8 ng/g (or less than 1 %) higher than that of low nitrogen

fertilizer use group, and 15 ng/g (or 1.5 %) higher than that of high nitrogen fertilizer use group. And the differences are statistically insignificant. Hence, it seems that there is no obvious relationship between nitrogen fertilizer applied and the expression of *Bt* toxin.

Second, we examine the relationship between expression of *Bt* toxin and phosphate fertilizer use. Similar as in the case of the nitrogen fertilizer, we reclassify the sample plots into three groups according to the amount of phosphate fertilizer applied: low

Table 2 Fertilizer application, saved seeds and expression of *Bt* protein toxin

	Fertilizer application (kg/ha)	Number of plots	Amount of *Bt* toxin, (ng/g)
Nitrogen fertilizer			
Low	0 to <100	483	986
Middle	100–200	240	994
High	>200	90	979
Phosphate fertilizer			
Low	0 to <50	478	979
Middle	50–100	279	993
High	>100	56	1,029*
Potash fertilizer			
Low	0 to <50	508	975
Middle	50–100	260	1,006*
High	>100	45	1,023*
Manure			
No		682	979
Yes		131	1,032**
Saved seed			
No		629	1,007
Yes		184	919***

***, **, * Significance at 1, 5 and 10 %, respectively. Statistical significance is based on one-tail t-test. For different chemical fertilizer use groups, the t-tests were conducted between middle and low fertilizer use groups, high and low fertilizer use groups, respectively

phosphate fertilizer use group (total phosphate fertilizer is up to 50 kg/ha), middle phosphate fertilizer use group (total phosphate fertilizer is greater than 50 kg/ha, but less than 100 kg/ha), and high phosphate fertilizer use group (total phosphate fertilizer is greater than 100 kg/ha). Different from that of nitrogen fertilizer use, there is a clear relationship between expression of *Bt* toxin and amount of phosphate fertilizer applied. As shown in the rows 4–6 of Table 2, the expression level of *Bt* toxin in plots with low phosphate fertilizer use is 979 ng/g, which is 14 ng/g lower than that of middle phosphate fertilizer use group, and 50 ng/g lower than that of high phosphate fertilizer use group, respectively. Further analysis shows that even though the difference between middle and low phosphate fertilizer use groups is statistically insignificant, the difference between high and low phosphate fertilizer use groups is statistically significant at the ten percent level.

Third, the relationship between expression of *Bt* toxin and the potash fertilizer applied is examined. As shown in Table 2, expression level of *Bt* toxin of plots with more potash fertilizer applied (i.e. middle and high potash fertilizer use groups) is significantly higher than that of plots with less potash fertilizer applied (rows 7–9). In fact, this result is not surprising since most of the plots were applied with compound fertilizers. In these compound fertilizers, usually the contents of phosphate and potash fertilizers are similar even though the content of nitrogen varies widely. In other words, plots with high phosphate fertilizer use are also those with high potash fertilizer use. As a result, the relationship between expression of *Bt* toxin and phosphate fertilizer applied is similar to the relationship between expression of *Bt* toxin and potash fertilizer applied.

Finally, we examine the relationship between the expression of *Bt* toxin and manure applied. Before the 1990s, China's farmers generally use a certain amount of manure to increase the long-term fertility of land. The manure usually comes from the livestock and poultry that farmers farm in their own backyards. However, as the economy develops and labor cost rises, many farmers give up backyards livestock and poultry farming, and turn to chemical fertilizer to keep or increase the crop yields. Hence, manure application has reduced sharply especially in recent years. In our samples, the total number of plots with manure application is 131, which is only 16 % of the total sample plots. Due to the fact that content of manure varies, we can not precisely quantify the active ingredients of nitrogen, phosphate and potash fertilizers respectively. Hence, we reclassify all the plots into two groups: plots with manure application and plots without manure application. As shown in Table 2, the expression level of *Bt* toxin of plots with manure application is 1,032 ng/g, which is 53 ng/g higher than that of plots without manure application (rows 10–11). The difference is statistically significant at the five percent level.

Table 2 also shows that expression level of *Bt* toxin is affected by saved seed. Planting saved seed is a quite common practice in China. According to our data, among the 813 sample plots, 184 plots were planted with saved seed (last two rows). The expression of *Bt* toxin of plots planted with saved seeds is 919 ng/g, which is 88 ng/g lower than that of plots planted with bought seeds. And the difference is statistically significant at the one percent level.

Table 3 Determinants of expression of *Bt* toxin in China's field production

| | Expression of *Bt* toxin | | |
	Model (1)	Model (2)	Model (3)
Nitrogen (kg/ha)	−0.001 (0.01)	0.017 (0.21)	0.007 (0.08)
Phosphorus (kg/ha)	0.447 (1.88)*		
Potassium (kg/ha)		0.847 (3.43)***	
Phosphorus and potassium (kg/ha)			0.358 (2.79)***
Manure dummy (1 = yes, 0 = no)	71.119 (2.83)***	73.290 (2.93)***	72.186 (2.88)***
Saved seed dummy (1 = yes, 0 = no)	−61.195 (2.59)***	−63.461 (2.70)***	−62.077 (2.63)***
Times of irrigation	3.414 (0.27)	3.657 (0.29)	3.244 (0.26)
No. of days after sowing	3.217 (1.69)	3.298 (1.74)	3.320 (1.75)
Soil quality (1 = high, 0 = others)	25.222 (1.65)*	25.799 (1.69)*	25.628 (1.68)*
Years of continuously planting cotton	−0.297 (0.05)	−1.331 (0.24)	−0.689 (0.13)
Village dummies	Yes	Yes	Yes
Variety dummies	Yes	Yes	Yes
Constant	1,527.024 (9.01)***	1,568.271 (9.45)***	1,531.980 (9.15)***
Observation	813	813	813
R-square	0.55	0.55	0.55

The figures in the parentheses are robust standard errors of estimates. ***, **, * Significance at 1, 5 and 10 %, respectively

Even though the above descriptive analysis seems to show that expression of *Bt* toxin may be related to whether the plots is planted with saved seeds, the amount of phosphate and potash fertilizers and manure application, and may not be related to the nitrogen fertilizer application, these unconditional relationships might be only false impression since the expression of *Bt* toxin might be affected by many other factors, such as varieties and environmental factors. Hence, to isolate the impact of farmers' farm management on the expression level of *Bt* toxin, we are going to set up a multivariate regression model in the following.

Estimation results

The estimation results are shown in Table 3. In general, all the three models appear to perform well in estimations. The R-squares are 0.55, a level of R-square should be considered as high for cross section data analysis. The signs of most of the coefficients estimated in all the three models are as expected. For example, the positive sign of *Sow* and *Soil* variables indicate that both earlier planting and good soil quality contribute to the high expression level of *Bt* toxin.

Consistent with the descriptive analysis above, the estimation results show that the estimated coefficients of nitrogen fertilizer are not significant in all the three models, which means that there is no clear relationship between nitrogen fertilizer use and the expression level of *Bt* toxin. This finding is contrary to previous studies based on laboratory or experimental fields (such as Wang et al. 1997; Zhou et al. 2000; Carlos et al. 2002; Yang et al. 2005). In the laboratory and experimental fields, the amount of nitrogen fertilizer applied might be firmly controlled below the optimal level, and increase of nitrogen fertilizer contributes to the nutrition status of the plant. Hence, increase the nitrogen fertilizer contributes to the expression of *Bt* toxin. However, in farmer's cotton production, as well as in other crop production, most farmers overuse nitrogen fertilizer (Zhang et al. 2006; Huang et al. 2008). Hence, at current nitrogen use level, inputting more nitrogen fertilizer does not contribute to the nutrition status of the plant and the expression of *Bt* toxin.

However, the estimated coefficients of both phosphate fertilizer and potash fertilizer are significant and positive (Table 3). As shown in model (1), the expression of *Bt* toxin increases by 44.7 ng/g if phosphate fertilizer is increased by 100 kg/ha. Similarly, the expression of *Bt* toxin increases by 84.7 ng/g if potash

fertilizer is increased by 100 kg/ha, as shown in model (2). Similar positive estimated coefficient is found in model (3) when sum of phosphate fertilizer and potash fertilizer is added in the function. In other words, our estimation results show that the expression of *Bt* toxin increases as the increase of phosphate fertilizer and/or potash fertilizer in cotton production in practice.

Similarly, the estimate results also show that application of manure has positive impact on the expression of *Bt* toxin. In all the three models, the estimated coefficients of manure application variable are significant and positive (row 5). According to this study, holding all the other variables as constant, the expression of *Bt* toxin in plots with manure application is about 70 ng/g higher than that of plots without manure application.

Finally, as what we have expected, express level of *Bt* toxin of plots planted with saved seeds is significant lower than that of plot planted with seeds bought in the market. As shown in Table 3, the estimated coefficients of saved seed dummy are all significant and negative (row 6). The estimation results show that if farmers planted saved seeds, the expression level of *Bt* toxin is about 60 ng/g, or about 6 %, lower than that of bought seeds (columns 1–3).

Discussion

In this study, we examine the expression of *Bt* toxin in farmer's fields and its determinants in North China Plain. Consistent with previous studies, this study shows that expression of *Bt* toxin is affected by its genetic characteristics of cotton varieties and environmental conditions. Moreover, the expression level of *Bt* toxin can decrease by more than 50 % from early growing season to late growing season.

More interestingly, different from previous studies based on the lab and experimental data, this study shows that expression of *Bt* cotton in farmer's agricultural production, has also been affected by seed quality and farmer's management. As in other developing countries, planting saved seed is a very common practice in China's cotton production (according to our data, 23 % plots were planted with saved seed). This study shows that the expression level of *Bt* toxin of saved seeds is significant lower than that of bought seeds.

Similarly, this study also shows that the expression of *Bt* toxin in agricultural production is significantly affected by farm management practices. In contrast to previous studies which showed that nitrogen fertilizer has a positive impact on the expression of *Bt* toxin (e.g., Yang et al. 2005), this study shows that nitrogen fertilizer application has no significant impact on the expression of *Bt* toxin in farmer's fields. On the other hand, the expression of *Bt* toxin has a positive relationship with phosphate fertilizer, potash fertilizer and manure application.

Result from this study has important policy implications. Unbalanced fertilizer use is quite common in China. Previous studies showed that farmers overuse nitrogen fertilizer but underuse phosphate fertilizer, potash fertilizer, and manure (Zhang et al. 2006). As the economic develops, especially as the increase of the labor cost, it is expected that farmers are going to use more chemical fertilizer, especially nitrogen fertilizer, and less manure. According to this study, balanced fertilizer use not only contributes to crop production, but also the expression of *Bt* toxin, hence leads to higher cotton yield and lower pesticide cost.

References

Adamczyk J, Sumerford D (2001) Potential factors impacting season-long expression of Cry1Ac in 13 commercial varieties of Bollgard cotton. J Insect Sci 1:1–6

Adamczyk J, Hardee D, Adams L, Sumerford D (2001) Correlating differences in larval survival and development of bollworm (Lepidopera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1Ac deltaendotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. J Econ Entomol 94:284–290

Baute T (2004) A Grower's handbook: controlling corn insect pests with *Bt* corn technology, 2nd edn. Canadian Corn Coalition, Canada

Carlos E, Robert D, John T (2002) Plant allocation to defensive compounds: interactions between elevated CO_2 and nitrogen in transgenic cotton plants. J Exp Bot 53:323–331

Coviella C, Stipanovic R, Trumble J (2002) Plant allocation to defensive compounds: interactions between elevated CO_2 and nitrogen in transgenic cotton plants. J Exp Bot 53:323–331

Cui J, Xia J (1999) The spatio-temporal distribution of *Bt* (*Bacillus thuringiensis*) insecticidal protein in *Bt* cotton. Cott Sci 11(3):141–146

FAO (2001) Action against undernutrition and poverty. Redirecting food assistance to those who need it most, 2001. In: The state of food insecurity in the World 2001, ISBN 92-5-104628-X. http://www.fao.org/docrep/003/y1500e/y1500e05.htm#P0_0. Accessed 6 June 2011

Gould F (1998) Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu Rev Entomol 43:701–726

Greenplate J (1999) Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. J Econ Entomol 92:1377–1383

Greenplate J, Penn S, Mullins J, Oppenhuizen M (2000) Seasonal Cry1Ac levels in DP50B: the Bollgard basis for Bollgard II. In: Dugger P, Richter R (eds) Proceedings of the Beltwide cotton conference, San Antonio, TX, 4–8 Jan. 2000, pp 1039–1041. National Cotton Council of America, Memphis, TN

Greenplate J, Mullins W, Penn S, Embry K (2001) Cry1Ac Bollgard varieties as influenced by environment, variety and plant age: 1999 gene equivalency field studies. In: Dugger P, Richter R (eds) Proceedings of the Beltwide cotton conference, vol 2. National Cotton Council of America, Memphis, TN, pp 790–793

Gutierrez A, Adamczyk J, Ponsard S, Ellis C (2006) Physiologically based demographics of *Bt* cotton–pest interactions II. Temporal refuges, natural enemy interactions. Ecol Model 191:360–382

Holt H (1998) Season-long monitoring of transgenic cotton plants-development of an assay for the quantification of *Bacillus thuringiensis* insecticidal protein. In: Proceedings 9th Australian cotton grower research association. Queensland, Wee Waa, Australia, pp 331–335

Huang J, Rozelle S, Pray C, Wang Q (2002) Plant biotechnology in China. Science 295(25):674–677

Huang J, Hu R, Pray C, Qiao F, Rozelle S (2003) Biotechnology as an alternative to chemical pesticides: a case study of *Bt* cotton in China. Agr Econ 29:55–67

Huang J, Hu R, Cao J, Rozelle S (2008) Training programs and in the field guidance to reduce China's overuse of fertilizer without hurting profitability. J Soil Water Conserv 63(5):165–167

James C (2012) Global status of commercialized Biotech/GM Crops: 2012. ISAAA Brief No. 44. ISAAA, Ithaca, NY

Kranthi K, Naidu S, Dhawad C, Tatwawadi A, Mate K, Patil E, Bharose A, Behere G, Wadaskar R, Kranthi S (2005) Temporal and intraplant variability of Cry1Ac expression in *Bt*-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera*. Curr Sci India 89:291–298

Mahon R, Finnergan J, Olsen K, Lawrence L (2002) Environmental stress and the efficacy of *Bt* cotton. Aust Cotton Grow 22:18–21

National Bureau of Statistics of China (2008) China statistics yearbook 2008. China Statistical Express, Beijing

Pray C, Ma D, Huang J, Qiao F (2001) Impact of *Bt* cotton in China. World Dev 29:813–825

Qaim M (2003) *Bt* cotton in India: field trial results and economic projections. World Dev 31(12):2115–2127

Qiao F, Wilen J, Rozelle S (2008) Dynamically optimal strategy to manage resistance to genetically modified (GM) crops. J Econ Entomol 101:915–926

Qiao F, Huang J, Rozelle S, Wilen J (2010) Natural refuge crops, buildup of resistance, and zero-refuge strategy for *Bt* cotton in China. Sci China Life Sci 53(10):1–12

Rao C (2005) Transgenic *Bt* technology: 3. Expression of transgenes. http://www.monsanto.co.uk/news/ukshowlib.php?uid=9304. Accessed 6 June 2011

Sachs E, Benedict J, Stelly D, Taylor J, Altman D, Berberich S, Daivs S (1998) Expression and segregation of genes encoding CryIA insecticidal proteins in cotton. Crop Sci 38(1):1–11

Shen P, Lin K, Zhang Y, Wu K, Guo Y (2010) Seasonal expression of *Bacillus thuringiensis* insecticidal protein and the control to cotton bollworm in different varieties of transgenic cotton. Cotton Sci 22(5):393–397

Wang R, Liu A, Li R (1997) *Bt* cotton R93-6 and nitrogen fertilizer requirement. Cotton Sci 24(4):16–17

Wen S, Dong H, Xin C (2007) Research progress in differential expression of *Bt* efficacy in transgenic cotton. J Henan Agric Sci 1:9–13

Xing C, Jing S, Cui X, Guo L, Wang H, Yuan Y (2001) The spatio-temporal distribution of *Bt* (*Bacillus thuringiensis*) insecticidal protein and the effect of transgenic *Bt* cotton on bollworm resistance. Cotton Sci 13(1):11–15

Yang C, Xu L, Yang D (2005) Effects of nitrogen fertilizer on the *Bt*-protein content in transgenic cotton and nitrogen metabolism mechanism. Cotton Sci 17(4):227–231

Zhang Y, Wu K, Guo Y (2001) On the spatio-temporal expression of the contents of *Bt* insecticidal protein and the resistance of *Bt* transgenic cotton to cotton bollworm. Acta Phytophy Sin 8(1):1–6

Zhang L, Huang J, Qiao F, Rozelle S (2006) Economic evaluation and analysis of fertilizer overuse by China's farmers. In: Zhu Z, Norse D, Sun B (eds) Policy for reducing non-point pollution from crop production in China. Environment Science Press of China, Beijing

Zhou D, Wu Z, Wang X, Ni C, Zheng H, Xia J (2000) Influence of fertilization and environmental temperature on the resistance of *Bt* transgenic cotton to cotton bollworm. J Anhui Agric Univ 27(4):352–357