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Effectiveness and chemical pest control of Bt-cotton in the Yangtze River Valley, China

1. Introduction

In China, genetically modified cotton (GMC) was first marketed in 1997, with varieties integrating Bt genes (Bt-cotton) to control some cotton pests, notably *Helicoverpa armigera*. By 2005, it was estimated that Bt-cotton is grown on about 60% of the total Chinese cotton-growing area (ISAAA, 2005), and close to 100% in areas with potentially major bollworm infestations (namely in the Yellow River Valley and Yangtze River Valley).

This acceptance of Bt-cotton could be explained by its cost-effectiveness due to a reduction in pesticide use (Huang et al., 2003; Huang et al., 2004; Pray et al., 2002). Three kinds of Bt-cotton varieties have been grown: varieties integrating the Monsanto Bt Cry 1Ac gene, varieties with the Chinese Bt gene[1] (Guo and Cui, 2004), and more recently varieties combining the Chinese Bt gene with the protease inhibitor CpTi gene (Cowpea Trypsin inhibitor). Most varieties currently grown in China have only the Chinese Bt-gene although there was a debate on the illegal use of Monsanto Bt-gene in Chinese varieties(Pray et al., 2006; Zou, 2003). The market share of the Monsanto gene varieties has dramatically fallen in recent years (10% in 2005 and presumably less since then). The relative share of GM varieties combining two genes has not yet been estimated.

The Chinese experience is a success story which has nevertheless recently been questioned, at least with respect to the Yellow River Valley, through papers accessible to the international community (CRICAAS, 2006; Lang, 2006), but documented much earlier in China (Yang and

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[1] This gene was constructed in 1992 by the team of Prof. Guo Sandui of the Biotechnology Research Institute, Chinese Academy of Agricultural Sciences. Various papers from this team mention this as being a Cry 1A gene, outcome of a gene synthesis. The first cotton varieties integrating this gene were experimented in 1995.

Guo, 2002). It is reported that the level of pest resistance is considered to be insufficient, the reduction in chemical control of bollworms seems to be counter-balanced by an increase in insecticides being applied to control other pests and farmers are also complaining about the substantial increase in Bt-cotton seed prices. This worrisome decline in the profitability of cotton growing likely underlies the Chinese Government's decision to implement a seed-subsidy program in 2007 (Liu, 2006; Wang and Lou, 2007; Yang, 2007), hence resuming subsidies, which had been discontinued in 1999, at the time when China was preparing its enter the WTO.

Factors underlying the effectiveness of Bt-cotton in China have not been analysed in detail. Are the varieties in the market really GMs? Is the gene expression correct? Is chemical control adapted for these varieties? Our paper is a contribution towards answering these questions. It is not easy to directly address these issues with up to 300 varieties being marketed, in 2005 (Lu et al., 2006), and very time-consuming to conduct a proper survey of farmers' cultivation practices, notably in the area of pest control, and to identify the varieties they use. Some authors have even underlined the difficulties encountered in identifying farmers' pest control practices through surveys in China (Pemsl et al., 2005a). The present study provides a more indirect methodology based on the results of a network of varietal experiments conducted in the Yangtze River Valley which have provided informative data on the issues addressed.

2. Materials and Methods

The varietal experiment network

The Yangtze River Valley varietal experiment network (YRVEN) dates back to 1950 and spans eight provinces, accounting for 35% of the cotton production in China in 2005. It currently assesses and recommends varieties adapted to local conditions through trials conducted at 22 locations (Table 1). The aim is to have varieties recommended for all or at least for several provinces of the network . A database was recently set up to help record and process results of more than 50 years of research. Results of data for the 2000-2006 period are considered in the present paper.

(Table 1: General information on the network trial locations)

The varieties in each trial are proposed by the institutions which have bred them. These generally have already been registered at local level and have been cultivated to some extent by farmers. So, the varietal performances in the network trials are quite indicative of what farmers can actually expect in the field. One variety served as a check in each trial. This was a non-GM variety until 2005, but it was replaced by a GM variety (XiangZa 8) thereafter. Seeds for all varieties tested were provided by the variety owners in quantities sufficient for two series of trials in case they had to be tested in a second year.

The experiment implementation

In all Chinese provinces, there are local research institutes at district and county levels which are mainly involved in implementing adaptive research, such as cultivation practices, crop protection, and plant breeding. The staff of these institutes are also responsible for implementing the YRVEN varietal trials, which were designed to identify varieties adapted to local conditions. Network collaborators were asked to apply local cultivation techniques. Decisions to apply chemical pest control were only made if required, according to the average pressure observed throughout the trial. With mostly Btcotton, the pest pressure observed was illustrative of the average Bt-trait effectiveness. Once decided, chemical sprays were applied on all varieties, regardless to their individual pest resistance status. Hence, chemical control was directed more to GM varieties and potentially less suitable for non-GM varieties.

In addition to the number and dates of sprays, additional information was required from 2004 to determine the pests targeted at each spray, the particular active ingredient targeted at a specific pest and when it was applied. The number of chemical applied is higher than the number of sprays, as a mixture can be applied in one spray to control several pests. More importantly, it has become possible to identify the date of the first bollworm control treatment.

Monitoring the GM status and bioassays

The GM status of the varieties has been monitored since 2003 by the Biotechnology Research Institute, Beijing, owner of the Chinese Bt gene (Xia and Guo, 2004). Until 2007, this Institute was the only organization authorized to assess the GM status of cotton varieties through ELISA tests. Varieties to be assessed are sown in 30 m^2 plots in May every year and ELISA tests are performed at the end of June on top leaves from plants at around 45 day post-emergence. The Bt-protein content is determined on the basis of absorbance values (in ng/g). The results of many experiments,indicate that a protein content of 450ng/g can be regarded as a threshold for good potential pest resistance.

Bioassays on the same varieties only began in 2004 at the Crop Protection Institute, Jiangsu Academy of Agricultural Science. The indoor and outdoor trials involved assays of cotton leaves picked from cotton plants grown in 20 m^2 plots per variety, which were generally sown around April 25. In addition to the varieties included in YRVEN, a pest-sensitive variety was sown to serve as a check in the bioassays.

Leaves were picked near the top of the cotton plants at the 4-5 true leaf seedling stage for the laboratory bioassays, in which six one-day old neonates are placed on two cotton leaves in Petri dishes. Five dishes were used per variety. The number of dead and alive bollworm larvae, and the extent of leaf damage were recorded after 3 and 5 days. All of these data, notably the mortality at days 3 and 5 for all varieties, were compared with the results obtained with the non-Bt check variety, according to the formula:

Adjusted mortality = (treatment mortality – check mortality)/(100-check mortality)×100

In the outdoor bioassays, nylon nets were placed over plots and 40 pairs of bollworm moths were introduced when the cotton plants had reached the squaring stage. The total number of squares and bolls attacked, the extent of their damage, and the number of living larvae were recorded 10 and 14 days later. The number of larvae surviving was compared with the check.

The larvae and moths needed for the bioassays were reared by the Crop Protection Institute at a constant room temperature of 26°C with 16 h of sunlight, from an initial collection of larvae from the field.. After about 30 generations, this population was considered quite sensitive to Bt toxin, although a few individuals had to be introduced into this population in recent years to preserve its vigour.

The names of varieties were coded by the YRVEN head agent, who was the only person who could match the codes and variety names after the analyses or bioassays had been implemented. Two non-GM varieties were integrated in the Bt-protein analysis and bioassays.

3. Results

Features of the tested varieties

The number of varieties submitted every year to the network for regional recommendation has risen to as high as 31 varieties (Table 2). These varieties no longer originate from public research institutions alone, as private companies are playing an increasing role. The principally GM varieties are also almost exclusively hybrids as these have stronger vigour, permit lower plant density and hence reduce labour requirement for the widely adopted technique of transplantation (Fok and Xu, 2007).

(Table 2: Basic information on the varieties tested in YRVEN)

Great variation and fluctuation in Bt-toxin production

The protein tests were effective for certifying the GM status of the varieties tested in YRVEN. No Bt-proteins were detected in the two non-GM varieties. The range of Bt-protein production in GM varieties (Figure 1) showed that except three varieties, the protein production was below 800ng/g. For most varieties, the protein production was above the 450ng/g threshold, thus suggesting a high pest resistance potential according to the norm retained in China. There were about 24 cases of production below this threshold (although there were 13 cases of protein production between 400 and 450ng/g), i.e. about 33 % of all tests carried out.

Figure 1: Distribution of varieties according to their Bt-protein production

Bt-gene expression is not only an issue between genetic background, as expression has fluctuated considerably for the same genetic backgrounds between years. Indeed, 10 varieties were tested in two subsequent years and their Bt-gene expression was evaluated. In spite of similar seeds from one year to another, Bt-protein production fluctuated substantially for more than 50% of the varieties which were tested in duplicate, revealing a quite substantial protein production gap of more than 200ng/g (about half of the threshold of 450ng/g for resistance effectiveness). We also processed data from the larger Yellow River Valley network in which the varieties tested were also mainly Bt ones but quite different to those submitted to the Yangtze River Valley network. The same protein production fluctuations were observed (Table 3).

(Table 3: Fluctuations in Bt-protein production for the same varieties)

Pest resistance confirmed but not perfect

Bioassay results were obtained for 56 varieties during the 2004-2006 period, and 10 of these were tested for two subsequent years, revealing some fluctuation in Bt-protein production, as mentioned above.

In the indoor bioassays, the observed larval mortality after 3 days was not sufficiently indicative of the mortality induced by Bt-toxin, which would require at least 5 days of monitoring. Varieties classified as pest resistant were resistant, especially at the very young cotton plant stage (4-5 real leaf stage). This was clearly indicated by the mortality observed in the indoor trials 5 days after the larvae were placed on cotton leaves: larval mortality was less than 60 - 80% only in 5% and 16% of the varieties tested respectively (Table 4).

The reduction in larval survival noted in the outdoor bioassays (implemented in Nanjing) was not correlated with the Bt-protein production measured (in Beijing) but the outdoor bioassay results were quite consistent with the indoor results, although the correlation between these results was not perfect (coefficient of determination of 33%). There was no reduction in larval survival in the field for around 10% of the GM varieties tested. For 10-20% of the other remaining varieties, the reduction was low (less than 60%). These figures are indicative of some pest-resistance effectiveness whose level is already considered to be below expectation in the Yangtze River Valley.

(Table 4: Bt-protein production and bioassay results)

Relatively frequent chemical control of cotton pests

The number of chemical controls and sprays[2] varied substantially between provinces and years. It cannot be considered that chemical control requirements diminished during the 2004-2006 period in any of the provinces considered while no changes were introduced in the spraying practices. Averaged across the 8 provinces, 14.5 insecticide treatments were applied in 8.2 sprays in 2006 (Table 5). Less chemical control was noted in three provinces, two of which (Anhui and Jiangsu) are partially connected to the Yellow River Valley where farmers reduced their chemical sprays to a greater extent after the advent of GM cotton (Pray et al., 2002).

(Table 5: Variation of the numbers of chemical controls and sprays in provinces)

Cotton plants still require up to five sprays against bollworms but with variation between provinces and years (Table 6). In addition to the well known *H. armigera* and *P. gossypiella*, the Asian Corn Borer (*Ostrinia furnacalis Guenee*) is another Lepidopteran pest, which is

[2] Each control is based on a particular active ingredient targeted at a specific pest at a specific time, so the number of chemical controls is higher than the number of sprays, as one spray can combine several active ingredients to control several pests.

reported to be a pest of increasing threat in the Yellow River Valley (He et al., 2006) where maize and cotton crops are grown.

More than two sprays are currently needed to control leaf-eating pests, but this category includes *Spodoptera litura* that the single gene Cry 1Ac has never controlled. It is reported that this pest no longer feeds only on leaves but attacks also squares and bolls, and whose chronic infestation is now regarded as a worrisome threat to cotton production (Guo et al., 2003; Li, 2004; Li et al., 2004; Qin et al., 2000; Russell and Deguine, 2006).

At all locations, sucking pests require more insecticide sprays than bollworms, as it was pointed out (Lang, 2006; Yang and Guo, 2002) and noted in two surveys carried out in Hebei and Jiangsu, respectively located in the Yellow and Yangtze River Valleys (Fok and Xu, 2007; Fok et al., 2005).

(Table 6: Chemical control patterns according to pest types)

4. Discussion

Reality and rationale of the wide diffusion of hybrid varieties

Since the year 2000, Bt and hybrid cultivars have dramatically increased their share among the varieties tested in the Yangtze regional network (Table 2). Bt-varieties increased from 30% in 2000 to 94% in 2006, while hybrid cultivars represented 100% of all the varieties tested in 2006. The dominance of hybrid varieties agrees with the full coverage of cotton area with these varieties in many provinces of the Yangtze River Valley (Xu and Fok, 2008).

The hybrid cultivars achieve a higher yield resulting from more numerous and heavier bolls compared to non-hybrid cultivars (Table 7). Hybrid and non-hybrid cultivars showed the same average value in terms of Bt-protein production during the 2003-2006 period, suggesting that the Bt gene used in creating hybrids is completely dominant.

Nevertheless Bt-cultivars did not show superiority for any of the criteria considered in the Yangtze River Valley, consistent with previous observations which have pointed out the low specific advantage of pest resistance by Bt-gene in the Yangtze River Valley (Xu et al., 2004).

The superiority of varieties which successfully pass through the regional testing, appears to be due mainly to improved lint quality criteria (lint length and "spinning quality"), as well as a slightly higher average yield (Table 7). However, this result is biased as non-hybrid cultivars contribute to the lower mean yield of the group of non-approved varieties. The less influence of yield criteria is illustrative of a situation where quite high yields have been obtained for many years.

(Table 7: Hybrid and GM effects on various recommendation criteria)

Efficacy threshold of Bt-toxin

In China, Bt-varieties are considered effective in pest resistance when the Bt-toxin production exceeds 450ng/g, as in 67% of the varieties tested during the 2003-06 period. The Bt-toxin production seldom exceeds 800ng/g. Since some authors have proposed the efficacy threshold of 1900ng/g of Cry 1Ac toxin, on the basis of research undertaken in India (Kranthi et al., 2005), could the low toxin production level being observed in the Yangtze River Valley network impact negatively on the pest resistance?

The low level of Bt-toxin production (relatively to what is reported in other countries for Cry 1Ac) is not specific to the Yangtze River Valley network. The levels we have reported are quite consistent with what has been found in previous studies. The maximum values were less than 600ng/g (Xia and Guo, 2004) and all values were below 600ng/g (except one value at 900ng/g) in another study (Xia et al., 2005). When the Bt-toxin production is reported from both the Monsanto and Chinese Bt-gene, the data is similar with at most 700ng/g (Wan et al., 2005), by using the ELISA kit provided by the American firm Agdia (Elkhart, IN). When the Bt-toxin production is controlled at the farmers' level[3], from cotton plants from seeds of Bt-varieties they have held back from previous season or they have bought, Bt-toxin

[3] Analysis was made by the Chinese Academy of Agricultural Sciences in Beijing.

concentrations seldom exceed 1000ng/g and 80% were below 800ng/g (Pemsl et al., 2005a). With reference to bioassays results, mortality ratios were quite substantial whatever the levels of the Bt-toxin production, this implies that the threshold retained in China does not seem to be too low. Nevertheless, the Bt-toxin production measured (in Beijing) is badly correlated to the results of bioassays (implemented in Nanjing); this observation implies that the figure of Bt-gene expression in one place is not a sufficiently good indicator of the pest-resistance efficacy in real conditions. This is consistent with the multitude of factors which impact on this efficacy (cf. infra).

The content of Cry1Ac toxin is reported to reach 6000ng/g in India and Australia. In India, depending on the plant parts and the period of analysis, the content has fluctuated from 50 to 5510ng/g (Kranthi et al., 2005). In Australia, a very wide range of Cry 1Ac toxin content has been obtained in various experiments to assess the effects of genetic background, agronomic practices and water or fertility stresses. The toxin concentration ranged from 270 to 6010ng/g, but most figures, even for varieties integrating the single Cry 1Ac gene, ranged from 1000 to 2500ng/g (Rochester, 2006).

The maximum values of Bt-toxin production measured in China are far much lower than in India and Australia, and their range is also smaller consequently. These lower values cannot be regarded as a factor of lower efficacy against the target pests when the results of bioassays are considered. Thus, the suggestion of an efficacy threshold of 1900 ng/g reported from India (Kranthi et al., 2005) cannot be applied to China.

Fluctuating expression of Bt-gene

During our research work, we observed that the expression of the Bt-gene in terms of production of Bt-toxin fluctuated a lot between varieties (Figure 1). This is consistent with the observation that genetic background is a major factor of fluctuation in Australia (Rochester, 2006). So far, in China, no systematic study has clarified the influence of genetic background on Bt expression.

Our results also demonstrate that Bt expression can fluctuate between years for a given variety (Table 3) with seeds of similar source and with the Bt-toxin concentration measured by the same laboratory. This result confirms that the expression of the Bt-gene, Cry 1A in our case, is sensitive to climatic factors as well as to agronomic factors (Rochester, 2006). This means that the Bt-protein production measured at one location one year cannot accurately reflect pest resistance ability everywhere and at all times.

Factors of lower pest resistance efficacy in the Yangtze River Valley

Our data is essentially related to the Yangtze River Valley, so does not allow comparison and thus confirmation that pest resistance is less than in the Yellow River Valley. However efficacy, although satisfactory, is far from perfect (Table 4) and raises the question whether pest resistance is sub-optimal in the Yangtze area.

One possible factor is the origin of the Bt-gene in the varieties used in the Yangtze River Valley. Based on studies (He et al., 2006; Wan et al., 2005), one can argue that the Monsanto Cry 1Ac is superior, and yet it is represented in only 4.7% of the total cotton area in Jiangsu province of the Yangtze River Valley in 2003, as opposed to 76.9% in Hebei Province of the Yellow River Valley (Xu and Fok, 2008). This indicates that the Monsanto varieties did not compete well against the Chinese varieties, subsequently making the Monsanto Bt-gene less attractive for integration into new varieties, even illegally[4] as it was observed in 2001 and

[4] It is quite possible that Monsanto Bt-gene has been illegally used in creating new varieties in the Yellow River Valley, but it is doubtful that this practice has persisted after the application of the Biosecurity Act and of the Plant Variety Protection Act (whose decree of application modalities were issued in 1999). After this implementation, breeders have to contract with the Biocentury Transgene Ltd to use the Chinese Bt-gene carried out by Prof. Guo Sandui's team, the only Bt-gene they can use. To formally register a new Bt-cotton variety, the breeder must provide a transgenic biosecurity certificate for which the Bt gene is identified through

reported in the Yellow River Valley (Pray et al., 2006). The argument of the superiority of the Monsanto Bt-gene is nevertheless flawed, at least in the Yangtze River Valley where the Monsanto varieties did not perform better than the Chinese varieties. Even in the Yellow River Valley, the superiority reported is debatable. Only very slight superiority has been observed for the Monsanto Cry 1Ac gene, in the control of the third generation of the Asian Corn Borer (He et al., 2006), late in the cotton crop cycle when complementary chemical control is needed. In other research, the expression of the Chinese Bt-gene was more variable along the cotton plant cycle and plant parts (Wan et al., 2005), but there is no difference when considering the case of top leaves till the early boll setting stage, organs and periods which are more crucial for the pest-resistance efficacy.

In China, the unique factor considered is the climate as high temperatures have reduced Bt-gene expression in experimental conditions (Rui et al., 2002; Xia and Guo, 2004). In reality, high temperatures might limit the Bt-gene expression but they can hardly explain the efficacy differential observed between the Yellow and the Yangtze River Valleys as temperatures are equally high during the cotton growing months.

Explaining factors have mainly to do with the specificities of the cotton growing techniques in the Yangtze River Valley. One of these factors is the widespread if not exclusive practice of transplantation (Fok and Xu, 2007). This technique is quite specific to China and notably to the Yangtze River Valley. Cotton seeds are sown in nursery and plantlets are transplanted when they reach the five-true leave stage. As yet, no research work in China has checked the influence of transplantating on the production of Bt-toxins, although plants are stressed and this could reduce the Bt-toxin production (Rochester, 2006).,

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DNA sequencing. Till 2007, the Bt-trait has been tested in Beijing by the research lab run by Prof. Guo Sandui, the owner of the Chinese gene.

The other specific factor in the Yangtze River Valley is the almost exclusive use of hybrid cultivars. Debates are ongoing as to whether the hybrid form of the varieties could induce a reduction in Bt-cotton effectiveness, with the argument put forward that the genetic resistance is derived from just one parent (Kranthi et al., 2005). This is nevertheless not consistent with the fact that Bt-genes are single dominant genes, so no reduction in resistance effectiveness has been observed in hybrid varieties (Xiao et al., 2001). The dominance might not be complete but this is not the case with the varieties considered, as we have noticed earlier.

The negative impacts on Bt-toxin production from using hybrid cultivars could be indirect ones. The plant vigour of hybrid cultivar has led to reduce plant densities in the Yangtze River Valley; this is the feature which has made hybrid cultivars so much attractive to farmers who implement transplantation because labour requirement is proportionally reduced. It is observed in Australia that the Bt-toxin production is reduced with low densities (Rochester, 2006). If this is true also in China, then the Valley efficacy differential is partly explained.

Another indirect negative factor linked to the use of hybrid cultivars is related to the lack of sufficient purity of hybrid cultivar seed, which is probably due to the constraints on manual hybrid seed production. Workers inevitably miss some flower buds when they implement the stamen elimination so some flowers are not hybridized and the seeds they produce are non-Bt cotton mixed with the hybrid seeds when the seed multiplication plots are harvested. The risk of collecting non-hybridized seeds is increased by rain which prevents access to the fields every day. Flowers which have been self-pollinated during the rainy days should be removed,, but some of them can be missed too. Thus purity of the hybrid seeds is not perfect, notably at commercial scale of their production. This shortfall might not be observed at the stage of regional varietal test because the variety owners will have provided the purest seeds.

Sub-optimal chemical control

Since the pest resistance of Bt-cotton is not total, variable larval survival rates are noted in the field, thus explaining why farmers might spray chemicals as a precaution. This precautionary behaviour implies a still high level of chemical control which has been observed but it was either related to the opportunistic behaviour of the extension staff who could make profit by selling insecticides (Huang et al., 2002) or to the lack of proper technical assistance to cotton growers (Pemsl et al., 2005a). Our results, suggest that the still high use of insecticides must also be due in part to the suboptimal effectiveness of Bt-cotton.

In China, it is generally acknowledged that *H. armigera* could undergo 4 to 5 generations during the cotton growing cycle and that GM cotton cannot control them all.Generally, the first generation coincides with the cotton plant seedling stage; the next generation appears at the cotton squaring stage; the third generation at cotton flowering; the fourth corresponds to the cotton boll setting stage while the fifth generation is at the beginning of boll opening. Surely GM cotton cannot control *H. armigera* up to the fourth generation at a period when Bt-gene expression has seriously slowed down (Yang and Guo, 2002). In the Yangtze River Valley, chemical control is generally recommended when high infestation levels are observed for the third generation of *H. armigera*.

In practice, we observed that at all the network experimental sites the first chemical control of *H.armigera* was always conducted before the *H. armigera* fourth generation. For the three year period of 2004-2006, 50-60% of the first spray was applied against the second generation and even earlier, particularly in 2005 when 37 of the sprays were directed at the first generation, probably because that year was warmer with possibly lower Bt gene expression.

The continued frequent chemical control against *H. armigera* could be explained by the fact that farmers still observe bollworms that have survived due to the insufficient genetic pest resistance of the cotton crops. This nevertheless highlights that farms habitually spray whenever bollworms are observed regardless of the infestation levels. Furthermore, so far no information is disseminated to help farmers adjust chemical control to threshold levels according to the bollworm populations.

5. Conclusion

This study provides further insight into the effectiveness of GM cotton use in China since the international community has become aware that its sustainability is under threat (Lang, 2006). It addresses this issue of effectiveness in the Yangtze River Valley, one of the two main regions where Bt-cotton is widely used in China. An indirect assessment approach was adopted for this study through processing of data collected within the Yangtze River Valley Varietal Experiment Network.

In this network, the status of all varieties classified as GM, with integration of the Bt-gene, was actually confirmed. However, this does not guarantee that all seeds of the same varieties sold to farmers were necessarily GM, i.e. there is sufficient information on the poor quality of seeds, and even fake GM seeds, sometimes supplied to farmers (Liu, 2006; Zhang et al., 2006). This situation prompted the launching of the quality seed subsidy policy in 2007.

It turned out that the expression of the Cry 1A gene substantially varied between genetic backgrounds and between two subsequent years for a few of these varieties. The efficacy of pest-resistance is confirmed but its level is not perfect, the mortalities of the *H. armigera* larvae in indoor and furthermore in outdoor bioassays are not always sufficiently high. This is consistent with the acknowledgement that the pest-resistance of Bt-cotton in the Yangtze River Valley is not so good, notably with reference to the Yellow River Valley. We suggest that this relatively lower efficacy could result from the transplantation technique which induces stresses to the cotton plant growth and development. We believe also that it is an indirect effect of the widespread use of hybrid varieties for which it is quite difficult to achieve pure enough hybrid seeds containing the Bt-gene.

Globally speaking, the Bt-cotton varieties being used cannot totally control bollworms even early in the growing season in the Yangtze River Valley. Surviving larvae will inevitably be observed, thus prompting farmers (or professional agents in charge of supplying technical assistance to farmers) to spray chemicals, regardless of the infestation level, i.e. the habit of total eradication seems to still predominate in the area of pest control. Bollworms seem to be sprayed more often than required and far earlier than necessary, i.e. against *H. armigera*. Farmers nevertheless are not to blame. No information is disseminated to farmers to inform on how to adapt chemical control according to infestation thresholds and to the bollworm generation number. This shortfall is unfortunately very likely to prevail in developing countries, hence justifying more attention to the institutional aspect in promoting GM varieties (Pemsl et al., 2005b).

From an economic viewpoint, due to the insufficient and variable pest resistance level of the Bt-cotton varieties released in the Yangtze River Valley, and partly to the lack of tailored complementary chemical control, farmers are paying high prices for Bt-cotton hybrids seeds which are not totally pest resistant while pest control costs are not reduced to the extent they might expect. These two phenomena undermine the cost-effectiveness of cotton production. An additional reason why chemical protection costs are not decreasing is that alternative pests are becoming an increasing threat due to the pest complex shift.

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Table 6: Chemical control patterns according to pest types

Province & year		Average number of chemical controls by pest types			
		borers	leaf-eater	Picker-sucker	pest from soil
Anhui	2004	1.0		1.0	1.0
	2005	3.0		4.5	1.0
	2006	1.5		7.5	1.5
Henan	2004	3.0		7.0	2.0
	2005	2.0		7.0	1.0
	2006	1.0		7.0	2.0
Hubei	2004	3.2	1.0	5.0	1.0
	2005	5.4	1.3	7.2	1.0
	2006	4.4	1.0	8.0	1.3
Hunan	2004	6.3	2.0	5.7	1.0
	2005	4.7	2.0	3.7	1.0
	2006	4.5	2.5	5.0	1.0
Jiangsu	2004	3.0	2.0	4.3	1.0
	2005	3.7	1.0	4.3	1.0
	2006	2.7	1.7	5.3	1.0
Jiangxi	2004	4.0	1.0	5.0	
	2005	6.0	2.0	8.0	
	2006	8.0	3.0	11.0	
Sichuan	2004	9.0		9.0	1.0
	2005	5.0		5.0	
	2006	15.0		8.5	
Zhejiang	2004	6.0	2.0	9.0	1.0
	2005	11.0	1.0	5.0	1.0
	2006	4.0	3.5	5.0	2.0

Note: borers are composed of *Helicoverpa armigera*, *Ostrinia furnacalis (Guenee)* and *Pectinophora gossypeilla(Saunders)*, leaf-eaters are composed of *Sylepta derogate Fabrilius* and *Spodoptera litura (Fabr.);* picker-suckers are composed of *Aphis gossypii*, *Tetranychus cinnabarinus, Adelphocoris suturalis (Jacovlev)*, *Lygus lucorum (Meyer-Dur)* and *Bemisia tabaci (Gennadius);* pests from soil are composed of *Agrotis ypsilon* and *Trachea tokionis(Butler).*

Table 5: Variation of the total numbers of chemical controls and sprays in provinces

	Number of chemical controls			Number of chemical sprays		
	2004	2005	2006	2004	2005	2006
anhui	3.0	8.5	10.5	3.0	7.5	6.5
henan	12.0	10.0	10.0	10.0	8.0	8.0
hubei	9.2	14.0	13.4	8.0	9.4	8.6
hunan	13.3	11.0	12.5	9.0	7.3	7.0
jiangsu	9.3	9.0	10.3	6.0	7.3	6.7
jiangxi	10.0	16.0	22.0	7.0	6.0	8.0
sichuan	18.5	10.0	23.5	8.5	7.5	11.5
zhejiang	18.0	17.0	13.5	10.0	10.0	9.0
Average	11.7	11.9	14.5	7.7	7.9	8.2

Table 4: Bt-protein production and bioassays results

Bt-protein production			<400 ng/g		400-600 ng/g		>600 ng/g	
			No. varieties	% Total	No. varieties	% Total	No. varieties	% Total
Indoor bioessays	Mortality, Day 3	<60%	2	67%	8	24%	9	47%
		60-80%	1	33%	11	32%	3	16%
		>80%			15	44%	7	37%
	Mortality, Day 5	<60%			1	3%	1	5%
		60-80%			4	12%	2	11%
		>80%	3	100%	29	85%	16	84%
Outdoor bioessays	Survival reduction, Day 14	No reduction	1	33%	2	6%	2	11%
		<60%			4	12%	4	21%
		60-80%			1	3%	1	5%
		>80%	2	67%	27	79%	12	63%

Table 3: Fluctuations in Bt-protein production for same varieties

		Yangtse River Valley network	Yellow River Valley network
Number of varieties analysed for protein content for 2 subsequent years		10	20
Protein content gap, ng/g	<100	3	3
	100-200	1	4
	200-400	3	8
	>400	3	5

Table 2: Basic information on the varieties tested in YRVEN

	No. varieties tested	% varieties from public institutions	% hybrid	% GM	No. varieties approved
2000	10	n.a.	50%	30%	1
2001	10	n.a.	60%	20%	1
2002	10	n.a.	70%	70%	1
2003	17	100%	71%	88%	3
2004	18	73%	71%	83%	2
2005	30	75%	90%	67%	3
2006	31	74%	100%	94%	4*

* 4 other varieties should be approved too after a new experiment

Table 1: General information on the network trial locations

Provinces	Cotton lint production, 2005		Number of trial locations
	in tons	in % China	
Anhui*	324 634	5.68	2
Henan*	677 000	11.85	1
Hubei	374 960	6.56	6
Hunan	197 511	3.46	4
Jiangsu*	322 660	5.65	3
Jiangxi	87 196	1.53	2
Sichuan	24 713	0.43	2
Zhejiang	21 566	0.38	2

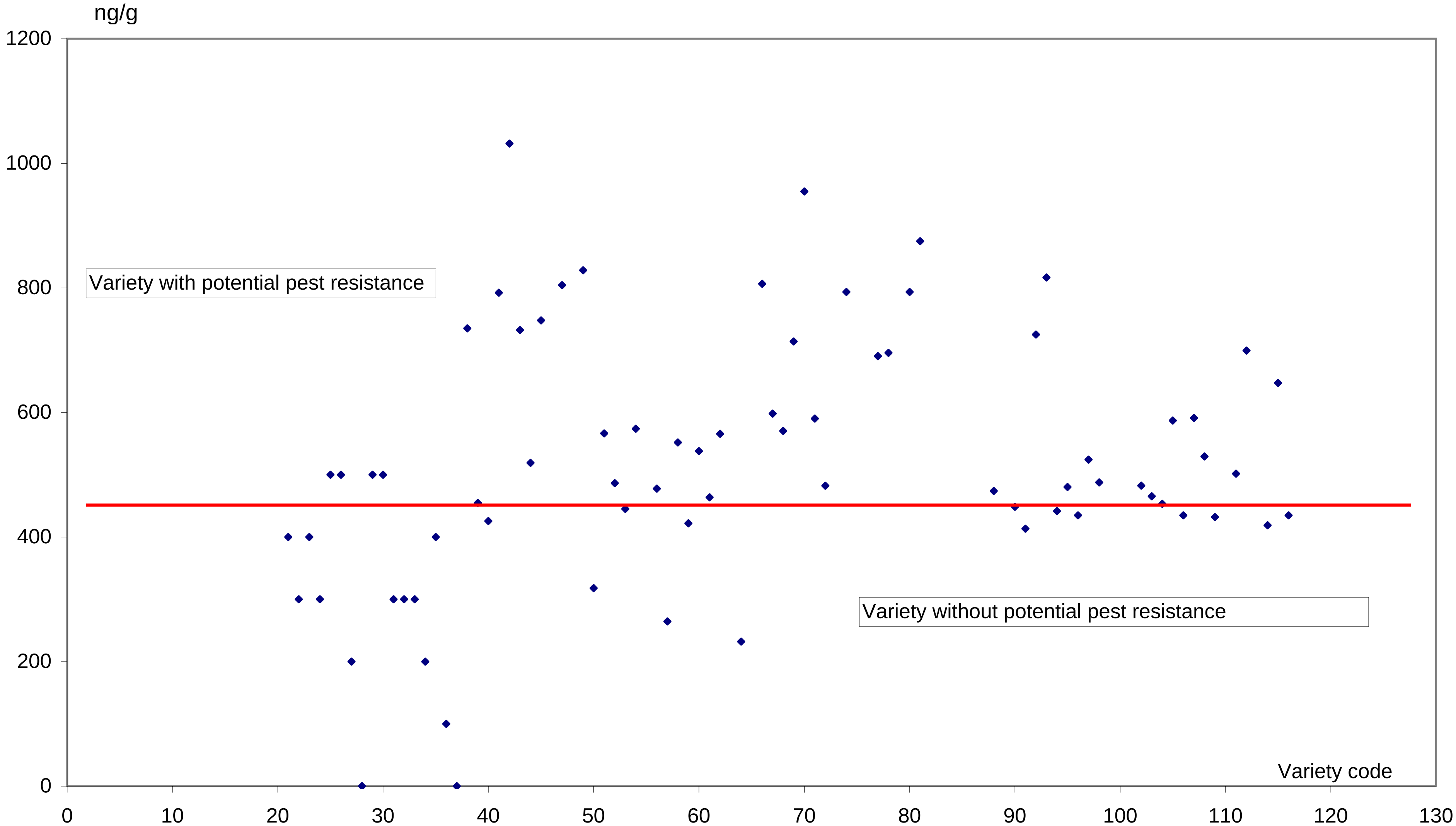
* only part of the related provinces belong to the Yangtze River valley. Cotton production is given for the whole province.

Table 7/ Hybrid and GM Effects on various recommendation criteria

		Seedcotton Yield kg/ha	Average Boll weight, g	No. bolls per ha	Bt-protein content, ng/g	Lint length mm	lint spin index
Hybrid	Yes	3 521	5.9	787 665	530	29.86	141
	No	3 057	5.3	793 327	530	29.88	141
	Probability. ANOVA	0.0001	0.0001	0.0101	0.5666	0.5835	0.6493
GM cotton	Yes	3 513	5.8	789 676	530	29.90	141
	No	3 219	5.7	786 643	0	29.76	140
	Probability ANOVA	0.0675	0.6840	0.1156		0.0489	0.2460
Variety approved	Yes	3 543	5.9	785 487	532	30.10	143
	No	3 368	5.7	790 278	534	29.76	140
	difference	175*	0.2**	4 792	-2	0.3*	3.2*

* t test Significant at 95%; ** t test Significant at 99%

Figure 1: Distribution of the tested varieties according to their Bt-protein content at 6-leaf stage



Variety code pertains to one variety and one year. The same variety tested during two years will appears in two distinct codes