1573 Detection of Contamination with Bt Transgene in Commercial Cottonseed of Argentinean Non-transgenic Cultivars

Ms. Ariela Gonzalez , EEA INTA Saenz Peña, Saenz Pena, Argentina
Mr. Olegario Manuel Royo , EEA INTA Saenz Peña, Saenz Pena, Argentina
Mrs. Maria Alejandra Simonella , EEA INTA Saenz Peña, Saenz Pena, Argentina
Ms. Elisa Martinez , EEA INTA Saenz Peña, Saenz Pena, Argentina

The rapid increase in acreage of transgenic crops in the last decade in Argentina and around the world, the concerns associated with this relatively new technology due to its potential impact on the environment and society, together with our own field observations suspecting transgenic contamination in commercial non-transgenic INTA`s cultivars in Argentina, determined that this study be carried out in order to have a measure of the country level contamination. An easy and economic methodology was developed for the purpose. It utilized a system of rearing the pest leafworm, *Alabama argillacea* Hübner (lepidoptera: noctuidae), very susceptible to the Bt toxin. Each test for each plant consisted of four replicates of leaf tissues from seedlings coming from seed samples taken from the gins where the commercial and certified cottonseeds of the non-transgenic cultivars were obtained. Evaluations of the neonate larvae status and the leaf tissue samples were carried out 24, 48 and 72 hours after they were put into contact. The methodology was calibrated with known Bt transgenic and non-transgenic leaf tissues. The percentage of plants expressing the Bt toxin over the whole country cotton area add up to 6.5 %. The analysis of the gins with contamination and the contaminated samples give figures of 71.4 % and 56.3 % respectively. These high values raise the concerns about better control measures at each stage of the production process of commercial cottonseed, and a need for a joint collaboration between governmental agencies of control and the seed industry.

Keywords: contamination, cottonseeds, transgenic

Introduction

In the GM Contamination Register Report 2005 released by GeneWatch UK and Greenpeace International (Anonymous 2005) a revision is presented of reported incidents in the public and scientific literature, of contamination, plantation, illegal releases of GMOs (genetically modified organisms) and negative side effects in agriculture since the GM crops were first grown commercially in 1996. A total of 39 countries were affected by one or more incidents. This is almost twice the number of countries that grow GM crops (21 countries). This demonstrates the potential widespread of GMO contaminations, and may represent only a sample of all real cases of contamination with GMOs, many of which are not detected or revealed because they are part of quality control systems (Anonymous 2005).

Data from this long report show that contamination can arise at each stage of development, from the laboratory to the plate (in foods). Also it states that no control system is perfect and that human error always produces incidents. Cases of identification mistakes, poor quality control, lack of knowledge, or inadequate control in the laboratories are reported. In incidents of seed contamination cross pollination, low standards of quality control, and lack of post-harvest segregation may play an important role (Anonymous 2005). The authors claim that public interest should prevail over strict confidentiality reasons, that seed importations from countries that grow GMOs should go through routine tests of control, and that international and national rules should be developed to allocate responsibilities for the economic, environmental, and health damage that may arise from GMO contamination (Anonymous 2005).

This new technology opens in agriculture a totally new perspective of unknown consequences in its impact on the environment. The magnitude is being debated about in many forums (Banchero 2003). In the case of transgenic crops important consequences should be expected; it involves profound genetic changes and very extensive crop acreages, and the impact on the environment could be far greater than that of the Green Revolution. From this arises the importance of detection of possible adverse effects for adequate diagnosis and anticipated correction. The great risk of transgenic crops resides in its success, because something successful expands rapidly (Banchero 2003).

With the steady adoption of GM crops around the world it is expected that the public will demand more controls and safety in quality and innocuousness of GM products, and in the products that are not wanted to be GM; and that world governments and trade will increase them. This work contributes to INTAs´ (National Institute for Agricultural and Livestock Technology of Argentina) advance in that sense.

In Argentina there are primitive landraces of *Gossypium barbadense* which may have received genes introgressions from the extensively cultivated *G. hirsutum* types. They are not wild but nevertheless their genomes have evolved long before Europeans came to South America. One of the risks that Bt cotton pose is the introgression of the trait into these germplasms. In their study Letourneau et al. (2003) conclude that there are high probabilities that cry genes of Bt crops will hybridize with wild plant populations which could in turn affect their fitness.

The objectives of this study were to evaluate and develop a control system for the genetic quality of non-transgenic commercial cultivars of INTA, oriented to the contamination with the Bt transgene released in the cotton growing region of Argentina, and to validate an easy and economic methodology of detection of the contamination.

Materials and methods

<u>Gins sampling</u>: in the year 2004 a random stratified sampling of more than 25% of the country gins (8 over about 20 to 25) was done. The sampling consisted of cottonseeds from the first multiplication category coming from certified fields in gins of several provinces of the Argentinean cotton growing region: Corrientes, Santa Fe, Chaco, Santiago del Estero, Formosa and Cordoba. Three samples of about 300 to 400 grams of seeds for two of the cultivars most grown in the country at that year, Guazuncho 2 INTA and Pora INTA, were taken. Samples were stored in refrigeration chambers at about 0°C.

<u>Cottonseeds plantation</u>: it was carried out in greenhouse in different pots where according to its size 50 to 100 seeds from one or two gins respectively were sown in two rows. Each pot had an identification with the seed origin. In this study the cultivar Guazuncho 2 INTA was selected, although for gins F and H Pora INTA was also analyzed. Gin F only had Pora INTA cottonseeds. Pots were irrigated regularly. No chemical or biological methods were used to control insect pests and weeds so that they would not interfere with the study. Fungicide was added in the last pots sown due to low temperatures to prevent damping-off infestation.

Evaluation methodology: a percentage index was obtained to evaluate Bt transgene contamination using a system of rearing neonate larvae of a common pest: leafworm (*Allabama argillacea* Hübner, lepidoptera: noctuidae) susceptible to the Bt toxin, where pupae are brought from infested fields and adults from them lay eggs in special cages (Royo et al 2005). This consisted of placing the neonate larvae, fed from cotton leaves in the lab, in a plastic grid were four replicates of leaf tissues in circular form (leaf discs) were placed on top of an agar-agar medium and covered with film so as to seal each plate and maintain moisture for the tissues.

Observations were registered at 24, 48 and 72 hours intervals after putting leaf tissues and neonate larvae into contact. Death of the four larvae in each of the four replicates for each plant and the leaf discs remaining almost intact was considered a positive (+) result which indicated that the plant was Bt transgenic. This normally occurred in the intervals of 24 to 48 hours after the experiment started. Death of the neonate larvae was evident by visible changes in appearance and motility. The method was checked with known Bt (Deltapine 404 BG) and non-Bt (Guazuncho 2 INTA) controls.

There was the need to have an efficient system of rearing the cotton leafworm, which was relatively easily attained though it required much effort and time for the production of larvae to be optimum. Also it was necessary to control for variables like moisture and temperature under adverse weather conditions, given that they were key factors at play on the pest biology (Polak et al. 2000; Polak et al. 2001).

Results and discussion

Table 1 displays data from the Bt and non-Bt controls. The plates without larvae column show mistakes done in the preparation of plates and represent cases where larvae escape from the plates or are trapped by the paper film when sealing plates. Though it is high, it represents the initial rates when preparing plates for controls, and they decreased considerably when the research advanced. For the Bt cultivar Deltapine 404 BG the percentage of survival cero meant no larvae survived ingestion of leaf tissues for all samples analyzed. Mortality rates do not reach 100 % due to the mistakes mentioned above. For the non-Bt cultivar Guazuncho 2 INTA the 3.9 % of mortality is associated with causes not related with the transgene expression but rather with the health status of larvae, e.g. fungal or virus attacks coming from the environment.

Bt transgene contamination by province: the total number of plants analyzed by province is considered in relation to the ones that came out as (+) transgenic. Table 2 displays these results.

As seen in Table 2 there are more data for the province of Cordoba. This is because seed samples were initially analyzed in 2005 for that province. Many cottonseed production fields are located in this area due to better weather and irrigation conditions for that purpose. If gin F is not taken into account for the sake of doubt because samples were not taken by authors, the province of Santiago del Estero would give a figure of **3.17 %** contamination (2/63 of the ratio number of transgenic plants/number of plants analyzed).

Following results are displayed at a country level both for a single cultivar and considering both cultivars together (Tables 3, 4 and 5).

Sampled provinces against provinces with contamination: of the 6 provinces where samples were taken 4 showed some degree of contamination, giving a **66.7 %** of Bt contamination result.

Gins with Bt contamination: 5 out of 8 gins studied had some degree of contamination, giving a figure of 62.5 %. If gin F is not considered the figure raises to **71.4 %.**

Samples analyzed: 19 samples across the different gins and provinces were analyzed, and 9 of them were found to be contaminated, giving the result of 47.4 % of samples contaminated. Without gin F it gets up to **56.3 %.**

In Argentina the cotton growing area could be divided into irrigated and dryland. Table 6 shows the percentages of contamination if results are grouped accordingly.

Although these results displayed through this study are only descriptive in nature, it says much about how rational is the world concern about the issue of GMO contamination, and the case of cotton transgenic contamination with the Bt transgene in Argentina could be considered a model, given the number of years of cottonseed production (almost 10) and the relatively small acreage grown, of what could go on happening if adequate control measures are not taken, specially for many countries that had recently started plantation of GM crops.

The figures reached what can be considered very high levels of contamination with GMOs. Most of the cottonseed production fields are grown in the drier west area of Argentina, and that is why the provinces at the irrigated area give the highest values of transgenic contamination in the gins, where they are ginned to produce the certified commercial cottonseeds. The provinces giving the 0 % contamination (Corrientes, Santa Fe) are in the east in the dryland area where acreages grown with cotton are relatively small compared to other provinces.

This methodology can be used until resistance of the pest leafworm to the Bt transgene is reached. It is very affordable and easily carried out, and it will be used to control levels of contamination in the basic stocks of INTA`s non-transgenic cultivars, probably until a DNA-based methodology much cheaper and accessible could be utilized. Previously no test for controlling contamination was used. Still it remains unknown how much of the contamination is caused by cottonseed mechanical mixtures, especially in the gins, and how much comes from cross-pollination.

Conclusion

Relatively high levels of contamination with Bt transgene has been demonstrated in this study, of a 6.5 % across the country, and reaching 12.15 % in the main area of increase of commercial cottonseed. These figures are based on samples of certified cottonseeds, but much of the cotton area is known to be planted with what is known as “white bags”, which are not certified seeds and therefore origin is not well known and mixtures are highly probable. The requirements of INASE (National Seed Institute) of segregation from other cotton crops for seed multiplication in Argentina are 100 meters with a barrier of other crop or bush or forest, and 200 meters without any barriers. Given the abundance of pollinators and these results, these segregation distances could even not be sufficient. This scenario shows that methods of detection of contamination and of control along the process are much needed, especially if consumers demand identification and segregation of GMOs and non-GMOs.

References

Anonymous. 2005. GM Contamination Report 2005. GeneWatch UK and

Greenpeace International. In: http://www.gmcontaminationregister.org

Banchero C. 2003. Desafíos agronómicos asociados a los cultivos

transgénicos. In: La difusión de los cultivos transgénicos en la Argentina. Pascale A.J. (Ed.). Editorial Facultad de Agronomía. Universidad de Buenos Aires. Pages 1-23.

Letourneau D.K.; G. S. Robinson and J.A. Hagen. 2003. Bt crops: Predicting effects of escaped transgenes on the fitness of wild plants and their herbivores. Environ. Biosafety Res. 2, pp 219-246.

Polak M. Et al. 2000. Incidencia de Alabama argillacea H. (lepidoptera:

noctuidae) en algodón y la relación entre su fenología, la del cultivo y las condiciones climáticas. In: Comunicaciones Científicas y Tecnológicas 2000, Universidad Nacional del Nordeste, Corrientes,Argentina.

Polak M.; Prause J.; Contreras G. y Caram G.E. 2001. Plagas del cultivo de

algodón Gossypium hirsutum (L.) (Malvales: Malvaceae) en relación a condiciones ambientales y sus estados fenológicos. In: Comunicaciones Científicas y Tecnológicas 2001, Universidad Nacional del Nordeste.

Royo, O.; Gonzalez A.; Simonella María A. And Martinez E. 2005. Detección

de la contaminación con el transgen Bt en las primeras multiplicaciones de cultivares convencionales de algodón de INTA. In: Proyecto Nacional de Algodón. Informe de avance N° 1. 2° Reunión Anual. Ediciones Instituto Nacional de Tecnología Agropecuaria. Sosa, M.A. y O. Peterlin (Ed). Pages 21-24.

Table 1: Mortality rates of *Alabama argillacea* neonate larvae in Bt and non-Bt control samples

Cultivar	Number of samples	Number of larvae in 4 replicates per sample	Number of larvae alive	Number of dead larvae	Plates without larvae	% Mortality	% plates without larvae	% survival
Guazuncho 2 INTA	38	152	138	6	8	3.9	5.3	90.8
Deltapine 404 BG	39	156	0	150	6	96.2	3.8	0

Table 2: Percentage of Bt transgene contamination by province in non-transgenic commercial cultivars

Province	Gin	Number of plants analyzed	Number of transgenic plants	Percentage of contamination
Corrientes	A	64	0	0
Santa Fe	B	62	0	0
Chaco	C,D	130	5	3.85
Santiago del Estero	E, F	119	2	1.68
Formosa	G	68	6	8.82
Cordoba	H	214	26	12.15

Table 3: Cultivar Guazuncho 2 INTA on a country level

Number of plants analyzed	Number of transgenic plants	Percentage of contamination
524	30	5.72

Table 4: All plants of both cultivars analyzed on a country level

Number of plants analyzed	Number of transgenic plants	Percentage of contamination
657	39	5.94

Table 5: All plants of both cultivars analyzed on a country level not considering gin F

Number of plants analyzed	Number of transgenic plants	Percentage of contamination
601	39	6.49

Table 6: Bt contamination percentages shown by irrigated and dryland areas

Cotton Area	Provinces	Number of plants analyzed	Number of transgenic plants	Percentage of contamination
Dryland	Santa Fe, Chaco, Formosa, Corrientes	324	11	3.39
Irrigated	Cordoba, Santiago del Estero	333	28	8.41