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Post-Market Monitoring Plans of Bt-176 in Spain: 1998 -2005

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Abstract

The first GM maize hybrids were registered in the Commercial Variety Register in Spain on the 26th of March, 1998. The registered hybrids, *Compa CB* and *Jordi CB*, were carrying the genetic modification identified as event “Bt-176”. This genetic modification expresses the Cry1Ab protein from *Bacillus thuringiensis* for protection against the insect pests: European Corn Borer *Ostrinia nubilalis* and the Mediterranean Corn Borer *Sesamia nonagrioides*. Since then, Spanish farmers have planted Bt-176 maize on their farms, growing to well over 22000 hectares annually (5% of total acreage). As part of the Spanish hybrid registration, the Spanish Authorities required that a post-market monitoring plan be implemented, which addressed several points. Syngenta presented a monitoring plan, which was further developed and carried out in collaboration with public research institutions in Spain. The monitoring plan has been in effect and results are now available. The results support the initial conclusions from the regulatory safety assessments, which were that Bt-176 maize is as safe as its conventionally bred counterpart, and indicate that Bt-176 maize does not have any unexpected negative impacts to the environment in Spain. The results of the post-market monitoring plan and our commitment to stewardship for Bt-176 maize in Spain are reviewed in more detail and we describe how these studies reinforce the conclusions from the initial regulatory assessments.

Introduction

The European Corn Borer (ECB) (*Ostrinia nubilalis* [Hübner]) and the Mediterranean Corn Borer (MCB) (*Sesamia nonagrioides* [Lefebvre]) first appeared in the records in 1902 (Garcia-Olmedo, 2003). They are the most damaging pests in Spain (Castañera, 1986), and are estimated to cause an average yield loss of 15% in some regions of high infestation. The average yield loss across the country is estimated to be within the range of 5-7% (Brookes, 2002). Conventional chemical control of these two major pests is particularly difficult due to the fact that they burrow inside of the plant, so Bt maize can be particularly effective and at the same time reduce traditional chemical inputs required on the maize plant.

The European Union approved the placing on the market of Bt-176 genetically modified maize according to Directive 90/220/EEC on 5 February 1997. Following this authorization at the EU level, the inscription in the Plant Variety Commercial Register of an EU State is required before the commercialization of any field maize hybrid seed for planting in the EU. Two hybrids, known as *Compa CB* and *Jordi CB*, were subsequently registered in the Commercial Variety Register in Spain on 26 March 1998. This registration allowed the commercialization of seeds from these registered hybrids in Spain. Syngenta has offered *Compa CB* for sale to Spanish farmers since 1998.

As part of the Spanish hybrid authorization, the Spanish authorities required that a post-market monitoring plan be implemented, which addressed the following points:

- An evaluation of the efficacy of the hybrids against target insects;
- A study on the potential development by corn borers of resistance to the Cry1Ab protein, in areas where Bt-176 maize is being grown to a significant degree;
- An evaluation of potential effects on the non-target entomofauna and on soil microorganisms, in fields cultivated with these Bt-176 maize varieties;
- An evaluation of potential effects on the population of bacteria in the gut flora of animals fed with these Bt-176 maize hybrids, in particular regarding the resistance to ampicillin;
- Indication of the area to be cultivated with conventional maize in relation to the area cultivated with these Bt-maize hybrids, in order to provide refuge for corn borers;
- A program for educating growers on proper use of the technology.

Syngenta presented a monitoring plan to Spanish Authorities. This plan was further developed and carried out in collaboration with public research institutions in Spain. Although the monitoring plan has been in effect since 2000, proactive studies were begun at the time of commercialization, and results are now available. These results indicate that Bt-176 maize does not have any unexpected negative impacts to the environment in Spain, indicating that it is as safe for the environment as its conventionally-bred counterpart.

Post-market monitoring for field resistance in target insects

Like any insect control method, target insects could potentially develop resistance to the insecticidal protein contained in Bt maize, which would affect the success of this powerful control tool. Therefore, resistance management strategies are being implemented to accompany the growing of Bt maize. In particular insect resistance is being carefully monitored, although to date, there has been no documented resistance of target insects developing in the field due to Bt maize (Tabashnik *et al*, 2003). Development of resistance can be detected through changes in susceptibility from baseline levels. If any susceptibility changes were to be detected, it would be possible to apply mitigation strategies.

To assess changes to susceptibility, it is necessary to first determine the baseline susceptibility of target pests to Cry1Ab protein. In 1998, González-Núñez *et al* (2000) determined the baseline susceptibility of four Spanish populations of MCB from Ebro, Albacete, Badajoz and Madrid, and two populations of ECB from Ebro and Badajoz, to Cry1Ab.

As part of the proactive resistance monitoring plan, field populations were monitored for resistance between 1999 and 2002. Additionally, laboratory selections with Cry1Ab protein were performed on MCB and ECB strains for eight generations.

Changes in susceptibility within a population were determined by comparing the lethal concentration of Cry1Ab protein in each year to the baseline susceptibility level. Farinós *et al* (2004) reported the results in 2004. The authors did not detect an increase in resistance in the MCB populations from Ebro, Albacete, and Badajoz, nor in the ECB populations from Ebro and Badajoz during the monitoring period. The susceptibility of the MCB population from Madrid fluctuated from year to year, but did not show a gradual trend toward higher tolerance levels. Laboratory selected MCB and ECB strains both showed marked increases in tolerance after eight generations of selection, but there were no consistent shifts in susceptibility for Spanish field populations of MCB or ECB after five years of Bt maize cultivation.

Interestingly, the authors noted a significant decrease in the tolerance level of the MCB population from Albacete from 1999 to 2000, and for the ECB population from Ebro between 2000 and 2002. As similar findings have been found in ECB field populations in the US, the authors suggest that these slight changes detected in susceptibility from year to year are the result of natural variability, rather than being indicative of any selection for resistance.

Thus, no increase in tolerance to Cry1Ab protein expressed by Bt-176 maize variety *Compa CB* has been detected in field populations of the target pests, *Sesamia nonagrioides* and *Ostrinia nubilalis*, during five years of commercial-scale cultivation.

Potential effects on non-target organisms

Bt-176 maize expresses Cry1Ab in the green tissues, pollen, and stalks, but not in the silks and kernels (Cannon, 2000). The specificity of Bt protein and the reduction of use

of conventional insecticides in Bt maize fields together are likely to result in a more favorable environment for the natural enemies of target pests. Studies performed by Pilcher *et al* (1997) in the USA, on small (25-50 m²) experimental plots, during two years of field evaluations, showed no detrimental effects on the abundance of coccinellids (beetles), anthocorids (insidious flower bug), and chrysopids (green lace wings) in Bt maize compared to non-Bt maize.

Additionally, Candolfi *et al* (2004) recently published one of the most comprehensive studies of its type on the impacts of Bt-176 on field arthropods in the EU. The authors examined the impacts of Bt-176 maize variety (*Occitan CB*), to its non-Bt isogenic maize variety (*Occitan*) which had been left untreated, or treated with either a conventional Bt spray (*Delfin*, Sandoz Agro AG) or a conventional pyrethroid insecticide (*Karate Xpress*, Zeneca S.A.). They monitored impacts on non-target arthropods in the soil, on the leaves, and flying in the crop area throughout the season by using the beating and funnel method (7 samples over the season) or pitfall traps (6 samples over the season). No effects of Bt maize were observed on the communities of soil dwelling ($P=0.05$) and non-target plant-dwelling arthropods ($P=0.05$), or on flying arthropods ($P=0.05$) throughout the season when compared to the control. Weak effects on two sampling dates during anthesis were observed of Bt maize on flying arthropods with lower abundance of adult Lepidoptera, flies in the Lonchopteridae, Mycetophilidae and Syrphidae families, and the hymenopteran parasitoids Ceraphronidae ($P\leq 0.05$). The authors suggested that lower ECB damage in the Bt maize could have resulted in the temporary declines in the presence of other taxa. The conventional Bt spray had short, but statistically significant effects on the plant dweller community. The pyrethroid insecticide had statistically significant effects in the short term on plant dwellers and long-term effects on soil dwellers.

As part of the monitoring program, de la Poza *et al* (2005) assessed the potential impacts of Bt-176 maize, variety *Compa CB*, compared to its non-Bt isogenic line, Dracma, on non-target invertebrates. Trials were conducted at two locations in Spain, one near Lleida and another near Madrid, over three years 2000, 2001, and 2002. The non-Bt isogenic line was either left untreated, or was treated with imidacloprid insecticide seed treatment (*Gaucha*, Bayer Crop Science). Except for the insecticide treatments, standard cultural practices in each area were used.

The composition and abundance of aerial predatory fauna were determined by visual surveys, and the activity of ground predators was determined by using pitfall traps.

The abundance of predators varied from year to year and between locations, but no clear tendencies related to Bt maize were recorded. Insecticide treatments reduced the anthocorid (insidious flower bug) numbers occasionally but no consistent effects were found for the rest of the predators. These data indicate that Bt maize is compatible with naturally occurring predators, which are common in Spanish maize fields and can contribute to reducing pest insect populations.

Potential impacts on soil microorganisms

The *bla* gene, encoding a TEM-1 β -lactamase (resistance to ampicillin), is included in Bt-176 maize, but is not expressed in the plant. Although no transfer of genes from plants to soil microorganisms has ever been documented to occur under typical field conditions, the possibility of such an exchange has been an area addressed by the risk assessments and several scientific studies. The European Food Safety Authority Scientific Panel on Genetically Modified Organisms expressed an opinion in 2004 that summarizes the concerns and conclusions of the risk assessment (EFSA, 2004a). The basis of the concern is the possibility that, if plant-associated soil bacteria were to acquire genes encoding antibiotic resistance from transgenic plants, they could potentially subsequently transfer the genes to human or animal bacterial pathogens. However, this process would require a sequence of coordinated events, involving the release and persistence of transgenic DNA in the environment, the uptake and integration in the genome of the recipient bacteria and the existence of a positive selective pressure towards the transformed bacteria. And in a subsequent opinion, the EFSA Scientific Panel on Genetically Modified Organisms stated that "...[T]here is no new scientific evidence, in terms of risk to human health and the environment, that would invalidate the risk assessments of genetically modified maize lines Bt-176... (EFSA, 2004b)."

The post-monitoring studies of Bt-176 in Spain examined the possibility of gene transfer of the *bla* gene to soil microorganisms in commercial maize fields. Badosa *et al* (2004) selected a field plot within commercial maize fields located in three locations in Spain (Zaragoz, Lleida, and Ablacete). The authors collected samples of soil, roots, and aerial plant materials from Bt-176 maize and its non-Bt isogenic cultivar (Dramca) during the growing seasons in 2000, 2001, 2002, and 2003. The samples were returned to the laboratory and bacteria were extracted from the samples. The extracted bacteria were cultured on agar petri dishes and the number of ampicillin-resistant bacteria was determined. The soil and root bacteria were further tested by PCR using primers specific to a region of the *bla* gene contained in Bt-176 maize.

In all bacterial samples (derived soil, root, and aerial plant) there were no significant differences in the population levels of ampicillin-resistant bacteria between Bt and non-Bt corn in any of the locations or during and of the samplings years. The PCR testing did not detect any transfer of the ampicillin resistance gene from Bt corn to the bacterial microbiota of the soil and root systems.

These data support numerous other scientific studies which have evaluated the possibility of transfer of genes from plants to soil bacteria since the development of the first genetically enhanced plants. The results of such studies indicate that impacts from the use of the *bla* gene in the development of GM plants would be insignificant against the background of already existing naturally resistant microorganisms (Scientific Committee on Plants, 2000) and would have no significance in terms of resistance (Swiss Co-ordination Group for Antibiotic Resistant Microorganisms, 2000).

Potential impacts on nutrition and gut flora of animals fed Bt-176 maize

Brufau *et al* (2001) performed further testing in order to specifically address the nutritional aspects of animals fed with Bt-176 maize compared to its non-Bt isogenic maize line, and to determine if Bt-176 maize could have any potential impacts on the ampicillin-resistance level of intestinal flora. The authors fed male leghorn chickens with Bt-176 maize, or its non-Bt isogenic maize (Dracma), for 32 days.

The nutritional equivalence of the Bt and non-Bt maize was examined. It was nutritionally equivalent to the isogenic non-Bt Dracma variety. Chicken daily weight gain, daily feed intake and feed to gain ratios, apparent metabolizable energy (AME), apparent metabolizable energy corrected by nitrogen retention (AMEn), and dry matter digestibility were not significantly different ($P>0.1$) between the Bt and non-Bt maize over the experimental time frame.

These data are consistent with those of other researchers, which have determined Bt-176 maize to be nutritionally equivalent to its non-Bt counterpart (for example, Brake and Vlachos, 1998; Aulrich and Flachowsky 2001; Aumaitre *et al*, 2002; and Clark and Ipharraguerre, 2001).

At the end of the experiment, the ceacum and samples from the breast, liver and blood were taken in order to determine the sensitivity of micro-flora to ampicillin and measure traceability of plant DNA. No significant differences were found in an ampicillin sensitivity test (Neo-Sensibats antibiograma dishes, 9mm diameter holes, 33 g of diffusible ampicillin per dish; $P>0.1$). The traceability tests detected the presence of plant DNA in all of the breast samples, and two liver samples, but in none of the blood samples.

Although fragments of plant DNA may be occasionally present, there is no indication that it poses any health risk. Einspanier *et al* (2003) reported that in such cases, there was no evidence that GM plant genes are specifically transferred or integrated in animal bodies. They further concluded that recent studies indicated that GM plant fed animal products are without any nutritional consequence.

Cultivation area

As part of the post-marketing monitoring plan, Syngenta is required to submit information on direct sales for Bt-176 maize.

Syngenta has voluntarily limited the amount of Bt-176 maize available for cultivation in Spain since it was commercialized in 1998. *Compa CB* was first planted commercially in 1998 when sufficient seed to plant 20,000 hectares was sold. Since 1998, the area planted with Bt maize has remained at this level (20,000- 25,000 hectares, equal to 4% - 5% of total Spanish maize plantings) because of a voluntary agreement from Syngenta Seeds to limit seed availability. The acreage of Bt-176 maize was reduced from average levels in 2001, due to limited seed supply that year.

Cultivation of Bt-176 maize area in Spain (data in Ha.)

	1998	1999	2000	2001	2002	2003	2004
Bt176	22.317*	24.952*	25.816*	11.550*	23.280*	26.085*	21.814
Total Bt	22.317	24.952	25.816	11.550	23.280	32.164*	58.000**
Total maize area (**)	519.000	396.063	437.559	577.500	423.272	478.760	483.148
% Bt176 Maize / Total maize area	4.3%	6.3%	5.9%	2.0%	5.5%	5.5%	4.5%
% Bt Maize / Total maize area	4.3%	6.3%	5.9%	2.0%	5.5%	6.7%	12%

*Source: MAPA

** Source: APROSE

In 1998 two Bt-176 maize hybrids, *Compa CB* and *Jordi CB* were registered in the Commercial Variety Register. From that date until 2003, the only genetically modified variety available on the market in Spain was the variety *Compa CB* (Bt-176) from Syngenta Seeds. In 2003, 5 new Bt hybrids (Event Mon810) from other companies (Limagrain, Nickerson, Monsanto, Pioneer) were registered. In 2004 another 10 Bt hybrids (1 Bt-176 + 9 Mon810) hybrids, each belonging to one Seed company, were inscribed in Spain.

In 2005, through the EU variety list, another set of hybrids registered in France became available to Spanish farmers. In total there are 21 Bt hybrids potentially available for commercialization in Spain.

Hybrids registered for potential commercialization in Spain

Bt maize variety	Event	Registration date	Seed company	Inscription at the EU list
Jordi CB	Bt176	B.O.E. 26 marzo 1998	Syngenta Seeds S.A.	
Compa CB	Bt176	B.O.E. 26 marzo 1999	Syngenta Seeds S.A.	
PR33P67	MON810	B.O.E. 11 marzo 2003	Pioneer Hi-Bred Spain, S.L.	17/09/2004
DKC6575	MON810	B.O.E. 11 marzo 2003	Monsanto Agr. España, S.L.	17/09/2004
Aliacan BT	MON810	B.O.E. 11 marzo 2003	Nickerson Sur, S.A.	17/09/2004
Aristis BT	MON810	B.O.E. 11 marzo 2003	Nickerson Sur, S.A.	17/09/2004
Brama	Bt176	B.O.E. 11 marzo 2003	Syngenta Seeds S.A.	
Campero BT	MON810	B.O.E. 16 febrero 2004	Advanta Iberica S.A.	17/09/2004
Cuartal BT	MON810	B.O.E. 16 febrero 2004	Arlesa Semillas, S.A.	17/09/2004
DKC6550	MON810	B.O.E. 16 febrero 2004	Monsanto Agr. España, S.L.	17/09/2004
Escobar	Bt176	B.O.E. 16 febrero 2004	Syngenta Seeds S.A.	
Gambier Bt	MON810	B.O.E. 16 febrero 2004	Nickerson Sur, S.A.	17/09/2004
Jaral BT	MON810	B.O.E. 16 febrero 2004	Semillas Fito, S.A.	17/09/2004
Protect	MON810	B.O.E. 16 febrero 2004	Koipesol	17/09/2004
PR32P76	MON810	B.O.E. 16 febrero 2004	Pioneer Hi-Bred Spain, S.L.	17/09/2004
Levina	MON810	Registered in France 1998	Pioneer Hi-Bred	17/09/2004
Elgina	MON810	Registered in France 1998	Pioneer Hi-Bred	17/09/2004
Bolsa	MON810	Registered in France 1998	Pioneer Hi-Bred	17/09/2004
Olimpica	MON810	Registered in France 1998	Pioneer Hi-Bred	17/09/2004
Novelis	MON810	Registered in France 1998	Coop de Pau	17/09/2004
DK513	MON810	Registered in France 1998	Monsanto	17/09/2004

Grower education

Syngenta is fully committed to the benefits that Bt-176 maize offers growers as an environmentally sustainable method of insect control.

In order to provide growers with the most up-to-date advice and information, Syngenta and its representatives are involved in research with the Spanish Agriculture Ministry and a leading Spanish agricultural research institute in order to develop specific recommendations for coexistence in Spain. As such, we provide a leaflet with each bag of Bt-176 maize that includes the traceability and labeling obligations with the seeds and harvest of Bt-176 maize, the Insect Resistance Management (IRM) recommendations,

and the coexistence recommendations for Spain. Syngenta actively engages growers individually and through grower organizations as partners in growing Bt-176 maize.

The entire seed industry has reached a common approach for the communication of these obligations and recommendations, which has contributed to the development of a common message for farmers. Farmers planting GM maize in Spain are advised by seed suppliers to plant some of their crops to conventional varieties to act as a refuge for the target species (corn borer) and hence to contribute to minimizing the possibilities of corn borers developing resistance to the Bt trait. Anyone planting over 5 hectares of Bt maize is advised to plant a non-Bt refuge, which should be equal to at least 20% of the total maize crop and should be planted close to the GM crop. These refuges can be in the form of strips (lateral, within or around the Bt crop) or as blocks between Bt crops.

Farmers are also advised about possibilities of adventitious presence of GMOs from their crops being found in non-GM crops and how best to minimize its occurrence. Advice relates to isolation distances and buffer zones that the farmer planting a Bt maize must secure between the GM and neighboring non-GM maize. 25 meter isolation distance or at least four rows of conventional maize planted between GM crops and non-GM crops are recommended.

Conclusions

Bt corn represents an environmentally sustainable way to control corn borer pests, therefore, to secure yield. There can be a significant economic return from growing Bt corn, with yields protected in years when there is a heavy outbreak of corn borer (Brookes, 2002), and there is evidence that Bt corn provides a form of protection to non-Bt corn by reducing the overall population of corn borers. Bt maize can also improve the quality of yield. Bt maize has been shown to have reduced levels of Fusarium fungal infections and their resulting chemical (Munkvold *et al*, 2000; Bakan *et al*, 2002), which are unhealthy for animals eating the infested grain.

The studies reviewed in this paper have been performed as part of the proactive and post-market monitoring program in Spain. Some key findings include the following. A baseline susceptibility survey indicates that there have been no changes in susceptibility of corn borers after eight years of Bt-maize cultivation in Spain. A 3-year farm-scale study, which assessed the potential impacts of Bt maize on predatory arthropods, has indicated that there are natural variations, but that Bt maize is compatible with naturally occurring predators. A thorough evaluation of the bacterial populations in the soil and guts of animals fed on Bt-176 maize demonstrate that none of the parameters measured were significantly different between Bt-176 maize and controls under the diverse conditions studied.

The data from these studies provide country-specific field data of large scale commercialized Bt-176 maize, which support and reinforce the conclusions from the original risk assessment and several years of research, which is that Bt-176 maize is as safe as conventional maize.

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