MONITORING REPORT

MON 810 cultivation

Czech Republic, France, Germany, Portugal and Spain

2005

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Introduction

Monsanto has developed an alternative to traditional insecticides for the control of lepidopteran insect pests, with reduced impact on the environment, by genetically modifying maize plants to produce the insecticidal protein Cry1Ab from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). These insect-protected maize plants, called MON 810, guard against foliage feeding and stalk tunneling from the European corn borer (ECB) (*Ostrinia nubilalis* (Hübner)) and the pink stem borer (*Sesamia nonagrioides*).

In April 1998, after a review of the risk assessment conducted for MON 810 in the notification (C/F/95/12/02, presented by Monsanto Europe S.A.) by France, acting as *rapporteur* country, by the competent authorities of the member states, and by the Scientific Committee on Plants, the European Union decided, in Commission Decision 98/294/EC, to approve the placing on the market of MON 810 in accordance with Directive 90/220/EEC (Commission Decision, 1998). According to this Decision, Monsanto S.A. committed to inform the Commission and the competent authorities of the Member States of the results of monitoring for insect resistance.

Decades of experience have taught entomologists that insect populations adapt, sometimes quickly, to even the best insecticides if the use of those insecticides is not managed appropriately. For this reason, as early as 1992 in the USA, Monsanto established an expert advisory panel composed of leading pest and resistance management researchers from academia, USDA-ARS, and university extension services to develop effective insect resistance management strategies for Insect-Protected maize.

Following this example, in the European Union, Monsanto has worked since 2001 to establish, with three other companies (Syngenta Seeds S.A.S., Pioneer Hi-Bred International, Inc., Dow AgroSciences), the "European Union Working Group on Insect Resistant Management" or EUWGIRM. This group developed an Insect Resistance Management (IRM) plan that enables concrete implementation of the management strategy described in Appendix III of the notification C/F/95/12/02 (Monsanto Company, 1995). This IRM plan (EU WG IRM plan, 2003) is based on the real experience acquired in world areas where MON 810 is grown, on results from research performed by scientists world-wide (including the EU) and on the scientific opinion on insect resistance published by the European Commission's Scientific Committee on Plants (SCP, 1999).

The total acreage planted in 2005 with *Bt* maize expressing the Cry1Ab protein was over 55 000 ha, in five EU countries (Czech Republic (270 ha), France (500 ha), Germany (270 ha), Portugal (780 ha)[1] and Spain (53 225 ha[2])). Spain started commercial cultivation of *Bt* maize in 1998 and therefore, it is the only country where monitoring insect for resistance is relevant.

MON 810 was commercially planted for the first time in 2003 in Europe. As last year report (Monsanto Europe S.A., 2005), this monitoring report describes the components and results of the **IRM plan** that was implemented in 2005.

[1] http://www.gmo-compass.org/eng/agri_biotechnology/gmo_planting/191.eu_growing_area.html
[2] http://ec.europa.eu/agriculture/events/vienna2006/presentations/ortega_en.pdf

In addition, Monsanto has undertaken a general surveillance monitoring program in 2005 on a voluntary basis, anticipating the mandatory request for post market environmental monitoring in all applications or renewal for deliberate release submitted under Directive 2001/18/EC and Regulation (EC) No 1829/2003 (including the future renewal of the MON 810 consent (Commission Decision, 1998)). The results of this **general surveillance** performed in 2005 are also reported and consist in two main elements, firstly a questionnaire to farmers that was design to assess unusual observation in the areas where MON 810 has been cultivated and secondly an assessment of the research work that led to peer reviewed publications published in 2005 and 2006 and that relates to MON 810 and its environmental safety.

Implementation of the IRM plan

The success of the IRM plan is ensured by the implementation of three key aspects. These are 1) refuge, 2) baseline studies and monitoring of the target pests, and 3) communication and education. These different aspects are reviewed in the following sections:

1) Refuge

According to the "Harmonised insect resistance management (IRM) plan for cultivation of *Bt* maize in the EU" (EU WG IRM plan, 2003), farmers planting more than 5 ha of MON 810 maize must have a refuge area planted with maize that does not express Cry1Ab and that corresponds to at least 20% of the surface planted with MON 810.

Many initiatives (*see* Section 3 "Communication and education") have been taken to explain to farmers the importance of implementing IRM measures. For cultural reasons, certain farming communities such as some of those in Spain are reluctant to accept "signed agreements" imposing particular agricultural practices. Moreover, seeds are usually sold through distributors and farmer cooperatives, with at least one step in the commercial chain, so signed agreement are very difficult to manage without direct sales between end-users and companies. As a consequence, the seed industry put a particular emphasis on the development of communication tools (*see* below).

In Spain, the effective implementation of refuges on farms was monitored at the end of the 2005 planting season through a survey sponsored by ANTAMA (Spanish Foundation supporting the use of new technologies in agriculture). At the same time the survey assessed the effectiveness of IRM communications programmes and the difficulties faced by farmers in implementing refuges. The survey was carried out in the Ebro Valley (Huesca, Lérida and Zaragoza), which is where most of the *Bt* maize that is currently planted in Spain is located. The survey involved 200 farmers who each planted more than 5 ha of maize (100 farmers planting *Bt* maize and 100 farmers planting conventional maize). The 100 farmers planting *Bt* maize collectively planted 2092.5 ha of maize, of which 1583.4 ha were *Bt* maize.

The result of the survey indicated that 100% of the farmers planting *Bt* knew the recommendation to plant a refuge. 93% of the farmers considered it clear enough but only 51% considered it easy to use. This survey also revealed that 56% of the farmers planted both conventional and *Bt* maize on their farm (conventional maize representing 24% of the total maize surface in farms planting *Bt* maize), with 49% of the farmers declaring that they had specifically implemented a structured refuge in their fields. In addition, 86% of the farmers who planted only *Bt* maize declared that the main reason was because of yield losses generated by *O. nubilalis* and *S. nonagrioides* in conventional maize.

It has to be mentioned that the excessive fragmentation of farms in small and disperse plots in some Spanish areas makes difficult the refuge implementation and although total farm could exceed 5 ha, plots which are part of it are smaller than 5 ha in many cases. Results from this survey reflect this situation. The average size of farms which are growing *Bt* and conventional maize is 24,1 ha whereas the average size of farms which are growing only *Bt* maize is 16,9 ha. Another relevant result is that 48% of the farmers who planted only *Bt* maize did it because of the small size of their plot.

In the context of the 2005 general surveillance, 132 farmers where surveyed across four countries where MON 810 was cultivated[3]. In the countries where MON 810 was planted for the first time in 2005, most of the farmers have indeed introduced a refuge. Seven of the total interviewed farmers (all from Spain) did not comply with the obligation of implementing a refuge. The reasons given were convenience or because neighbours' conventional maize field could serve as refuge. *Bt* maize has been introduced in Spain in 1998, but an extended communication on IRM essentially started in 2003 with the introduction of MON 810. The non compliance to the use of refuge might be linked to the Spanish history of *Bt* maize introduction, when the surface planted with *Bt* maize was limited and remained below 6% of the total maize market. Farmers planting *Bt* maize also tend to rely on their neighbours' conventional maize fields as refuge.

Although the *Bt* maize planting in Spain has not exceeded 12% of the maize area to date, it is important to pursue the effort in educating the Spanish farmers on the necessity to implement refuge, and it has been reiterated by different actions which have been put in place by the seed industry for the 2006 cultivation year (*see* Section "Communication and education"). This key element is also being followed up in other countries where the technology has been introduced more recently.

[3] Germany was not included in this study. Instead a separate survey was undertaken by the German Federal Biological Research Centre for Agriculture and Forestry (BBA), maize breeders and statisticians (Wilhelm *et al.*, 2004).

2) Baseline and monitoring studies

a) Baseline studies

Baseline studies with Cry1Ab performed in Spain (three major regions (Ebro Valley, the centre of Spain and Extremadura-Andalusia) where insect pressure would justify the use of MON 810 maize) by the research group led by Dr. [redacted] and Dr. [redacted] (Department of Plant Biology, Centro de Investigaciones Biologicas, CSIC) and presented in last year report (Monsanto Europe S.A., 2005), revealed no difference in susceptibility among populations of ECB or among populations of *S. nonagrioides* collected in those three regions (González-Núñez *et al.*, 2000) prior to the introduction of *Bt* maize in Spain.

In 2005, upon request of Monsanto, additional baseline studies have been conducted within Europe.

The research group of Dr. [redacted] and Dr. [redacted] established the baseline susceptibility to Cry1Ab within the French and Portuguese field populations of *S. nonagrioides* and *O. nubilalis*. *S. nonagrioides* was collected from the Midi-Pyrénées (France) and Bajo-Alentejo (Portugal) areas while *O. nubilalis* was sampled from the Bajo-Alentejo area (Ortego, 2006a; Ortego, 2006b).

The susceptibility to Cry1Ab of those species lies within the range obtained with Spanish population of these species (Farinos *et al.*, 2004) and no resistance to Cry1Ab has been observed in any of the populations.

BTL Bio-Test Labor GmbH (Sagerheide, Germany), also initiated a baseline study which covers four major European maize growing regions: South West France, Rhine valley/Southern Germany, Northern Germany/Western Poland and Czech Republic/ Western Slovakia. This study is still in progress and results will be communicated at a later stage.

b) Monitoring for insect resistance

As reported last year (Monsanto Europe S.A., 2005), the group of Dr. Pedro Castañera and Dr. Felix Ortego has performed monitoring for *O. nubilalis* and *S. nonagrioides* resistance to Cry1Ab across the three above-mentioned Spanish regions since 1999, the date of the commercialization of the *Bt* maize *Bt* 176 from Syngenta that also expresses a Cry1Ab protein (Farinos *et al.*, 2004).

Over the last two growing seasons (2004 and 2005) the same group performed monitoring for *O. nubilalis* and *S. nonagrioides* resistance to Cry1Ab expressed in MON 810 (Ortego, 2005; Ortego, 2006c). Samples were collected from the Ebro Valley, Albacete, and the Extremadura-Andalusia region (Spain).

The results of the monitoring studies performed with *O. nubilalis* and *S. nonagrioides* in 2004 have been presented in last year's report (Monsanto Europe S.A., 2005).

The monitoring studies performed with *O. nubilalis* and *S. nonagrioides* collected during the 2005 season did not reveal any resistance to Cry1Ab among the regions. The susceptibility to the Cry1Ab of the *O. nubilalis* and *S. nonagrioides* populations lies within the range obtained with populations of these species collected from the same geographical areas during the 1999-2002 period (Farinos *et al.*, 2004).

The lower susceptibility of the population from the *S. nonagrioides*-Ebro Valley to Cry1Ab observed in 2004 and reported in Monsanto Europe S.A. (2005) was further investigated in 2005. Results with the same population did not confirm those results and suggested the results obtained in 2004 were probably due to inappropriate storage of the toxin used in the study (4°C instead of -20°C).

In parallel with the resistance monitoring on corn borers populations through field collection and lab bioassays, seeds companies are following up and attending to occasional complaints by farmers about lack of efficacy, which could indicate resistance development. So far, no complaint related to lack of efficacy of a MON 810 field has been reported and results from the ANTAMA survey (*see* point 1) showed 96% of the farmers who plated *Bt* maize in 2005, were very or enough satisfied with the overall results.

3) Communication and education

An extensive grower education program is essential for the successful implementation of the IRM plan.

As mentioned in last year's report, each purchaser of *Bt* maize receives a technical user guide that contains the latest information on the growers' IRM obligations. The user guide requires farmers to implement IRM measures, including refuge planting. Examples of those documents can be found in Annex 1.

The grower education programme has been communicated within all seed companies that sell maize expressing Cry1Ab proteins.

In 2005, in order to measure the effectiveness of this education programme, a Spanish survey funded by ANTAMA, was performed in the Ebro Valley (Huesca, Lerida and Zaragoza), which is the main *Bt* maize area in Spain. The survey involved 100 farmers who each planted more than 5 ha of *Bt* maize and who collectively planted 2092.5 ha of maize. 100% of the farmers answered that they were made aware of the fact that they are required to plant a refuge.

In 2006 planting season, especially in Spain, additional emphasis was given to refuge implementation by:

1) Reinforcing communications about IRM implementation in all sales tools (leaflets, brochures, catalogues, hybrids guides on pack):

   Examples:

   - APROSE guide attached in each MON 810 bag (leaflet common to all companies in Spain);
   - Technical Guide on MON 810 "Guía Técnica YielGard®";
   - Hybrid variety Guides (attached DKC6575 hybrid but one per registered hybrid has been created, delivered in the bag or through distributors)
   - Dekalb catalogue (page on regfuges)

2) Talking directly to farmers (presentation used by our sales team/distributors in all farmers talks)

3) Developing a YieldGard®[4] testimonials DVD (including IRM requirements) for farmer talks

4) Displaying "ad hoc" posters during field days

5) Emphasize the presence of "real refuges" in our demo trials in order to educate and train farmers planting *Bt* maize

6) Using a bag tag which displays the IRM refuge requirement

7) Reinforcing IRM implementation during the two days Sales Team meeting

8) Advertising IRM in magazines and trade fairs during the 2006 campaign

9) Articles on IRM or on Good Agricultural Practice with *Bt* maize published in four key Ag Magazines

10) Sending a letter with a brochure to the 2 700 maize farmers that are included in the Monsanto data base and located in ECB areas and encouraging our licensees to do the same

11) Incorporating the IRM training and communication to farmers in the Commercial Policy under "Activities objective"

Illustration of some of those actions is presented in Annex 2.

Further surveys are planned for the 2006 season to assess the effectiveness of these initiatives.

[4] YieldGard® is a registered trademark of Monsanto LLC.

General surveillance

Monsanto has undertaken a general surveillance monitoring program in 2005 on a voluntary basis, anticipating the mandatory request for post market environmental monitoring in all applications or renewal for deliberate release submitted under Directive 2001/18/EC and Regulation (EC) No 1829/2003 (including the future renewal of the MON 810 consent (Commission Decision, 1998)).

The objective of the general surveillance is to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the environmental risk assessment. It is largely based on routine observation and implies the collection, scientific evaluation and reporting of reliable scientific evidence, in order to be able to identify whether unanticipated, direct or indirect, immediate or delayed adverse effects might been caused by the placing on the market of a genetically modified crop in its receiving agronomic or non-agronomic environment.

General surveillance is focused on the geographical regions within the EU where the GM crop is grown, and is taking place in representative environments, reflecting the range and distribution of farming practices and environments exposed to GM plants and their cultivation.

Where there is scientifically valid evidence of a potential adverse effect (whether direct or indirect), linked to the genetic modification, then further evaluation of the consequence of that effect should be science-based and compared with baseline information. Relevant baseline information will reflect prevalent agricultural practice and the associated impact of these practices on the environment. In many cases it may not be possible to establish a causal link between a potential adverse effect and use of a particular GM crop

The general surveillance performed in 2005 consists in two main elements, firstly a questionnaire to farmers that was designed to assess unusual observation in the areas where MON 810 has been cultivated and secondly an assessment of the research work that led to peer reviewed publications published in 2005 and 2006 and that relates to MON 810 and its environmental safety.

1) Questionnaire

Farmers are the closest observers of the cultivation of the GM crops and already collect information on the cultivation and management of their crops at farm level. Therefore they can give details on GM plant-based parameters (referring to species/ecosystem biodiversity, soil functionality, sustainable agriculture, or plant health) and on background and baseline environmental data (*e.g.* soil parameters, climatic conditions, general crop management data *e.g.* fertilisers, crop protection, crop rotations and previous crop history). Additionally farmers may give empirical assessments which can be useful within general surveillance to reveal unanticipated deviations from what is common for the crop and cultivation area in question, based on their historical knowledge and experience.

A questionnaire addressed to the GMO cultivating farmers is a monitoring tool that is specifically focused on the farm level. EFSA explicitly considers questionnaires a useful method to collect first hand data on the performance and impact of a GM plant and to compare the GM plant with conventional plants (EFSA, 2006). The questionnaire approach has also proven its applicability with other industries, *e.g.* the pharmaceutical industry.

A farmer questionnaire has been developed as the key tool for monitoring of MON 810 (*see* Annex 3). It was inspired by the experimental questionnaire develop by the German Federal Biological Research Centre for Agriculture and Forestry (BBA), maize breeders and statisticians in Germany. Its questions were refined to be too easily understood by farmer and not to be too burdensome. Also it had to be pragmatic enough to take into account real commercial situations.

Farmers have been asked for their observations and assessment in and around MON 810 cultivated fields in comparison to a baseline, this being their own historical local knowledge and experience. This general surveillance for MON 810 focused on the geographical regions within the EU where MON 810 was grown in 2005 (Czech Republic, France, Portugal, Spain). It also was performed in areas reflecting the range and distribution of farming practices and environments exposed to MON 810 plants and their cultivation. This allows for cross-checking of information indicative of an unanticipated effect, and the possibility to establish correlations either by comparing questionnaires between regions, or associating answers to observations made by existing networks, such as meteorological services (weather conditions) or extension services (pest pressure).

A subset of farmers in Spain (65) and almost all farmers in Czech Republic (five), France (38) and Portugal (24) were asked to fill in the questionnaire. The field representatives assisted the farmers in filling in it.

It contained five sections:

1. Personal data
2. The farm and farm activities
3. *Bt* maize specific measures
4. Observations related to MON 810 maize in the field
5. Observations after harvest of MON 810 maize

Section 1 gave the coordinates of the farmer which are treated as confidential information.
Section 2 was to obtain information on the size of arable land and the proportion of maize (*Bt*/non *Bt*/other GM maize).
Section 3 referred to the specific measures to follow when cultivating *Bt* maize (training, label recommendations on seed bags, use of refuge).
In section 4 farmers were asked to fill in their observations on fertility, use of fertilizers, pests and diseases, weed and volunteer, use of herbicides, crop rotation and crop history, use of crop protection products and proximate field surroundings. The comparison was always made to conventional maize. Furthermore, space for the recording of additional observations was provided.

Section 5 related to post-harvest observations (level of control against target pests, anything unusual, use of MON 810 for livestock). Other remarks, if any, related to the cultivation of MON 810 could be added.

This questionnaire has been used by Monsanto and its licensee, Pioneer Hi-Bred International, Inc.,which are the two main MON 810 seed providers.

The analysis of the 132 questionnaires being surveyed in 2005 on the cultivation of MON 810 maize did not indicate any adverse effect. The full report is presented in Annex 4. This first set of data is entered in a database which will be completed year after year with new entries constituted by responses to questionnaires

The farm questionnaires will be distributed, completed and collated annually. Reports will be prepared also on an annual basis, except in case of adverse findings that need immediate risk mitigation, which will be reporter as soon as available.

Learning from this 2005 experience has allowed Monsanto to develop a new and improved version of the questionnaire that will be used in the 2006 season. (*see* Annex 5).

2) Peer reviewed publications on the safety of MON 810 and/or Cry1Ab published in 2005 and 2006

An important source of information on MON 810 is the extensive independent research that is performed by scientists with a wide range of expertise such as insect and microbial ecology, animal toxicology, molecular biology or chemistry. More than 30 publications related to MON 810/Cry1Ab were published in peer reviewed journals in 2005 and 2006. Those references related to MON 810 or pure Cry1Ab were obtained by running a search using the search engine ISI Web of Knowledge™ (search terms: MON 810 or MON810; Transgenic maize or corn; *Bt* maize or corn; Genetically modified maize or corn; Cry1Ab and other).

These publications and other public research efforts reinforce our knowledge of MON 810, its safety and commercial performance. The list of those peer reviewed publications can be found in Annex 6.

The data available, overall, indicate no detrimental effects of MON 810 and/or Cry1Ab.

Conclusions

The commercial planting of MON 810 in Europe has been accompanied by a rigorous Insect Resistance Management (IRM) plan, centred on three major elements: refuge implementation, monitoring, and farmer education.

No issues related to Insect Resistance Management were experienced in 2005. Nevertheless, improvements in the IRM plan need to be achieved. The main objective for 2006 is to increase the percentage of farmers implementing refuges in their fields, especially in Spain. For this purpose, the process of educating retailers and farmers has been further reinforced in 2006, and the messages on the necessity of implementing refuges strengthened.

Monsanto and the seed companies marketing maize expressing the Cry1Ab protein have been operating together to establish an IRM programme that is adapted to the EU agricultural landscape, and will continue to work closely together to assess its implementation and subsequently build on those learnings.

Regarding general surveillance, the results of the analysis of the first set of questionnaires did not identify any potential adverse effects that might be related to MON 810 plants and their cultivation. 2006 questionnaire has been improved based on the experience acquired with 2005 questionnaire. In addition, 2005 and 2006 peer reviewed publications confirmed the negligible potential of MON 810 and/or Cry1Ab to cause adverse effects.

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