Harmonised insect resistance management (IRM) plan for cultivation of *Bt* maize (single insecticidal traits) in the EU

- September 2013 -

EuropaBio

The European Association for Bioindustries

EuropaBio Monitoring Working Group

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Summary

With the introduction of *Bt* crops, concerns have been raised about the possible development of insect resistance that could deprive growers of the benefits of *Bt* crops and *Bt* microbial preparations. This concern has been addressed pro-actively in a number of countries by the implementation of insect resistance management (IRM) plans to delay the potential development of pest resistance and to enable the timely detection of changes in pest susceptibility allowing remedial actions to be put in place. In the EU, with the introduction of *Bt* maize cultivation in 1998 in Spain, research programmes were established to monitor the potential development of insect resistance. In 2001, with the introduction of Directive 2001/18/EC, IRM plans became mandatory. Given that different *Bt* maize varieties targeting the same insect pests were commercialized in the EU at that time (*Bt*176 and MON 810) and other varieties were under review for approval (TC1507, *Bt*11), developers of the technology united efforts and proposed a common IRM plan. The purpose of this harmonized plan was to develop and use common methodology to establish the baseline susceptibility of European corn borer (ECB; *Ostrinia nubilalis*) and Mediterranean corn stalk borer (MCB; *Sesamia nonagrioides*) to Cry1Ab and Cry1F endotoxins and to monitor the potential development of resistance following the cultivation of these *Bt* maize varieties. The plan was implemented in 2003 and has been in place since then. Despite fourteen years of use of *Bt* maize in the EU and high adoption rates of the technology in some areas, no decreases in the susceptibility of ECB or MCB to Cry1Ab have been detected. This suggests that the implemented harmonized IRM plan was effective. In the EU as well as worldwide, no field resistance to any Cry1Ab and Cry1F-containing event or formulation has been observed in any species of *Ostrinia* or *Sesamia.* However, one of the elements described in the plan was to maintain it updated in view of the findings and in view of new scientific information. Since the first implementation of the harmonized IRM plan there have been some updates in the regulatory framework. Additionally, there has been a large amount of data generated by the previous plan and in the scientific literature, and experience has been gained from IRM plans established in other regions. Taking together all this information, the EuropaBio Monitoring working group has now updated the IRM plan.

This document describes the updated IRM plan including the key elements to follow and the rationale behind the recommendations. The current IRM plan is in line with the recommendations and guidance provided by the current regulatory framework.

Abbreviation/definition of technical terms

Area	Area is defined as a geographical zone where a given crop is typically grown following similar agronomic practices and is isolated from other areas by barriers that might impair an easy exchange of target pests between those areas
Bt	*Bacillus thuringiensis*
Bt maize	Maize plants expressing *Bt* Cry proteins
Cry protein	Crystal protein derived from *Bt*
EC_{50}	Effective concentration: the concentration, which affects 50% of a test population after a specified exposure time
EC_{99}	Effective concentration: the concentration, which affects 99% of a test population after a specified exposure time
ECB	European corn borer
Endotoxin	Toxic molecule associated with the outer membrane and cell wall of bacteria
GM	Genetic modification
Grower	Individual responsible for seed purchasing and planting
IPM	Integrated pest management
IRM	Insect resistance management
MCB	Mediterranean corn stalk borer
MIC_{50}	Moulting Inhibition Concentration (*i.e.*, the concentration/dose of substance that is estimated to inhibit molting of 50% of the test organisms)
Field resistance	Field resistance is defined as a genetically-mediated ability of a target pest to survive on one or more commercial line(s) of *Bt* corn under field (or near fieldsuch as greenhouse) conditions. This ability may be conferred to heterozygotes, but must be conferred to homozygotes. It is demonstrated by an ability of the insect to feed and complete development on *Bt* corn. Fitness costs (e.g. delayed development, reduced competitiveness, or fecundity) may be associated with the resistance.
Lab resistance	Lab resistance is defined as a genetically-mediated reduction in sensitivity of a target pest to *Bt* toxins, either in artificial diet or leaf-disc bioassays. Such resistance may be observed as an increase in population MIC_{50}, or as enhanced growth or survival at a discriminating concentration, compared to a known susceptible line. Such resistance does not necessarily confer the ability to develop on *Bt* corn plants in the field.

Population resistance Population resistance occurs when a large portion of a pest population is field-resistant and causes the *Bt* maize to fail to confer economic control of the population.

1 Introduction

Maize is an important crop in the European Union (EU) and infestations of European corn borer (ECB; *Ostrinia nubilalis*) and Mediterranean corn borer (MCB; *Sesamia nonagrioides*) can result in considerable crop damage and yield loss. In Spain for example, the losses in maize production due to these insect pests can be as high as 15% in areas of high corn borer pressure. Given the biology of corn borers, the use of conventional insecticides is not very effective as chemical sprays cannot reach the boring pest larvae. Genetically modified (GM) maize plants have been developed to control these pests. These GM plants express *Bacillus thuringiensis* (*Bt*) proteins, such as Cry1Ab and Cry1F, that provide specific control of lepidopteran pests, by consumption of the proteins when feeding on the maize, and are very effective against ECB and MCB. The plants are commonly known as *Bt* maize.

Bt is a Gram-positive bacterium capable of producing large crystal protein inclusions that have insecticidal properties. The efficacy and specificity of *Bt* strains and individual toxins produced by *Bt* isolates are such that a large number of insecticidal products based on this bacterium and/or its toxins have been developed and sold commercially since the late 1950's. Historically, *Bt* has been considered a safe option for pest control and it has often been the preferred pest control method in Integrated Pest Management (IPM) programmes.

Using modern biotechnology, the genes coding for specific *Bt* toxins were isolated in the 1980's and introduced into various crop plants to provide insect protection. Such insect-protected crops now represent an important new management tool to control crop damage and losses due to insect pests. In addition, the use of insect protected crops provides important benefits to growers, society, and the environment (Gómez-Barbero, 2008; Brookes and Barfoot, 2009; MacIntosh, 2009; Park *et al.*, 2011; Brookes and Barfoot, 2012).

Maize plants expressing *Bt* proteins for pest control were first registered for commercial use (deregulated) in the USA in 1996. Currently, genetically modified (GM) maize containing an insect protection trait (as such or in combination with herbicide tolerance) is the second most widely planted GM crop, with approximately 43.3 million hectares commercially grown worldwide in 2011, mainly in the USA, Canada, Argentina, Brazil and South Africa (James, 2012).

With the introduction of *Bt* crops, concerns were raised about the possible development of insect resistance that could deprive growers of the benefits of *Bt* crops and *Bt* microbial preparations. The development of resistance in insect pests to pesticides is a well known phenomena and the development of insect resistance to *Bt* crops is accepted in the scientific community as a possibility. Anticipating this concern, biotechnology companies have been working with academic experts, regulators and growers to design and implement proactive insect resistance management plans for *Bt* crops. As a result of the implementation of these IRM plans no field evolved resistance in ECB or MCB have been reported, neither in the EU nor on a global scale. Only isolated reports of field evolved resistance to other pests have been cited globally (Kruger *et al*., 2012). However, these reports have been linked to non-compliance by farmers with IRM recommendations, and in particular non-compliance with refugia requirements (Kruger *et al*., 2012). This shows that the implementation of good IRM practices and customer education and resistance monitoring have slowed the spread of field evolved resistance (Van Rensburg, 2007; Kruger *et al.*, 2009).

In the EU, *Bt* crops were introduced in 1998 and the adoption has steadily increased every year. In 2011, six EU countries (Spain, Portugal, Czech Republic, Poland, Slovakia and Romania) planted a total of 114,508 hectares of *Bt* maize, 26% more than in 2010, with Spain growing 85% of the total in the EU with an adoption rate of 28% (James, 2012).

With the introduction of *Bt* maize cultivation in 1998 in Spain, research programmes were established to monitor the potential development of insect resistance. In 2001, with the introduction of Directive 2001/18/EC (EC, 2001), IRM plans became mandatory. Given that different *Bt* maize varieties targeting the same insect pests were commercialized in the EU at that time (*Bt*176 and MON 810) and other varieties were under review for approval (TC1507, *Bt*11), developers of the technology united efforts and proposed a common IRM plan. The purpose of this harmonized plan was to develop and use common methodology to establish the baseline susceptibility of European corn borer (ECB; *Ostrinia nubilalis*) and Mediterranean corn stalk borer (MCB; *Sesamia nonagrioides*) to Cry1Ab and Cry1F endotoxins and to monitor the potential development of resistance following the cultivation of these *Bt* maize varieties. The plan was implemented in 2003 and has been in place until now. Despite fourteen years of use of *Bt* maize in the EU and high adoption rates of the technology in some areas, no decreases in the susceptibility of neither ECB nor MCB to Cry1Ab have been detected. This suggests that the harmonized IRM plan was effective for Cry1Ab. However, one of the elements described in the plan was to maintain it updated in view of the findings and in view of new scientific information. Since the first implementation of the harmonized IRM plan there have been some updates in the regulatory framework. Additionally, there has been a large amount of data generated by the previous plan and in the scientific literature, and experience has been gained from IRM plans established in other regions. Taking together all this information, the EuropaBio Monitoring working group has now updated the IRM plan.

This document describes the updated IRM plan including the key elements to follow and the rationale behind the recommendations. The current IRM plan is in line with the recommendations and guidance provided by the current regulatory framework. The goal of the IRM plan is to detect resistance far enough in advance of population resistance to allow adequate time for the confirmation and characterization of resistance, and to take steps to reduce the likelihood or extent of product failure. The plan has been designed so it is effective, balanced and practical for the growers of *Bt* maize.

2 Scope of the plan

The goal of the IRM plan described in this document is to detect resistance far enough in advance of population resistance to allow adequate time for the confirmation and characterization of resistance, and to take steps to reduce the likelihood or extent of product failure. The IRM plan includes timely detection of changes in pest susceptibility to Cry proteins and remedial actions in case of any confirmed development of resistance. A timeframe of two to two and a half years between initial resistance detection and implementation of an appropriate remediation plan is considered adequate. The transformation events currently included in the proposal are presented in Table 1 and are further in this document referred to as *Bt* maize.

Table 1. Proteins and transformation events currently included in the harmonised IRM plan

Transformation event	OECD unique identifier	Protein	Notifier
***Bt*11**	SYN-BT∅11-1	Cry1Ab	Syngenta
MON 810	MON-∅∅81∅-6	Cry1Ab	Monsanto
1507	DAS-∅15∅7-1	Cry1F	Pioneer; Mycogen/DAS

The main insects targeted by the plan are the European corn borer and the Mediterranean corn stalk borer, as shown in Table 2.

Table 2. Insects targeted by the harmonised IRM plan

Common name	Abbrev.	Scientific name	Family
European corn borer	ECB	*Ostrinia nubilalis* (Hubner)	Crambidae
Mediterranean corn stalk borer	MCB	*Sesamia nonagrioides* (Lefebvre)	Noctuidae

3 Approach and rationale of the plan

3.1 Regulatory framework in the European Union

The updated IRM plan proposed by the EuropaBio Monitoring working group has taken into account the recommendations and guidance provided by the current regulatory framework.

Directive 2001/18/EC (EC, 2001) was the first to establish that notifiers should develop and submit a monitoring plan together with the notification for placing on the market of a genetically modified (GM) crop. The design of the post-market environmental monitoring plan (PMEM) was outlined in Annex VII of this Directive. The objectives of the monitoring plan were described as: (1) to confirm that any assumptions made regarding the occurrence and impact of potential adverse effects of the GMO or its use in the environmental risk assessment (ERA) are correct, and (2) 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 ERA. In line with the regulatory framework, Annex VII to Directive 2001/18/EC was later supplemented by the Council Decision 2002/811/EC providing further guidance on the objectives, general principles and design of monitoring plans. More recently, the European Food Safety Authority (EFSA) GMO Panel updated its scientific opinion on the PMEM of GM plants (EFSA, 2011a), following the opinion on the ERA of GM plants (EFSA, 2010).

3.2 Practical experience from IRM plans implemented around the world

The first country to introduce *Bt* maize for commercial cultivation was the USA in 1996. The rapid success of this technology and the high rates of adoption led to the US Environmental Protection Agency (EPA) to view *Bt* crops as a “public good” and to adopt measures to protect the technology. The US EPA now requires the monitoring of insect resistance development to *Bt* crops as a condition of registration (US EPA, 2001; MacIntosh, 2009). Developers of the technology in collaboration with experts from academia, USDA, EPA and the Agricultural Biotechnology Stewardship Technical Committee (ABSTC) developed a harmonized industry IRM plan for *Bt* maize (ABSTC, 2003). This plan is based on the high-dose/refuge strategy (also further addressed in this document below) and comprises all the elements required by US EPA[1], such as the use of structured refuge requirements, grower agreements and resistance monitoring programs for Cry1Ab and Cry1F *Bt* maize (Siegfried *et al.*, 2007). To date, despite the high level of adoption of *Bt* maize in the USA during almost two decades, there are no reports of field-evolved resistance in populations of ECB to Cry1Ab or Cry1F (Tabashnik *et al.,* 2003; Siegfried *et al.*, 2007; Tabashnik *et al.,* 2009; Head and Greenplate, 2012).

In Argentina the first *Bt* maize product was approved in 1997. *Bt* maize was initially introduced with a variety of voluntary IRM practices, but in 1999, building upon the experiences in the USA, a joint industry IRM plan was developed in collaboration with experts from academia and the Instituto Nacional de Tecnología Agropecuaria (INTA). The harmonised IRM plan was also based on the high-dose/refuge strategy and proposed the use of a 10% refuge requirement. The proposal of this refuge size was based on knowledge of the biology of the local target pest and on grower behaviour. In particular, it was noted that the presence of abundant alternative hosts for the target pests justified refuge sizes smaller than in the USA for target pests that were otherwise similar in their biology. The IRM plan also included the development of baseline susceptibility measurements for the target pests, the creation of standardised educational literature for growers and the use of regular surveys to assess grower compliance with the requirements. The joint industry IRM plan was accepted by the regulatory agency Comisión Nacional Asesora de

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[1] http://www.epa.gov/oppbppd1/biopesticides/pips/bt_corn_refuge_2006.htm#websites (Accessed July, 2012).

Biotecnología Agropecuaria (CONABIA) and implemented. To date, there are no reports of field resistance of target pests to Cry1Ab or Cry1F.

Similar approaches have been followed in other countries such as Canada, South Africa and Brazil, where harmonized industry plans have been developed in collaboration with experts from academia and regulatory authorities, to protect the technology. All these IRM plans for *Bt* maize crops are based on the high-dose/refuge strategy, although the size of the refuge may vary depending on the biology and ecology of the target pest in that country. In South Africa, there have been some cases of insects other than ECB or MCB developing resistance to *Bt* maize in the field, however, these cases have been linked to poor implementation of IRM practices among customers; further customer education efforts and monitoring efforts have slowed the spread of resistance (Van Rensburg, 2007; Tabashnik *et al.,* 2009; Kruger *et al.*, 2012).

3.3 Practical experience from the previous IRM plan implemented in the EU

In the EU, *Bt* maize has been cultivated since 1998 in Spain. Research programmes supported by the Spanish authorities and the industry harmonized IRM plan that has been in place since 2003 have provided a large body of information on baseline susceptibility of the target pests populations in different EU countries, on susceptibility levels after continuous exposure to *Bt* maize and on the ecology of the pests (See Section 3.4 below for a summary). In addition, since the only *Bt* maize currently cultivated in the EU is MON 810, Monsanto, in compliance with current regulatory requirements, has submitted annual monitoring reports to the European Commission since 2005 (the two most recent reports: Monsanto, 2010 and Monsanto, 2011 are accessible through the European Commission's website[2]). These reports provided information on the findings of the IRM plan in place for Cry1Ab, including baseline susceptibility data for ECB and MCB and susceptibility data following exposure to MON 810 (See Section 3.4 below for a summary of the findings). The two most recent reports have now been reviewed by EFSA and scientific opinions have been adopted (EFSA, 2011; EFSA, 2012). These scientific opinions have been taken into consideration while updating this IRM plan.

One of the key conclusions of all this research is that no susceptibility shifts have been established for field populations of MCB or ECB after fourteen years of *Bt* maize cultivation, showing that the IRM plan in place has been effective.

3.4 Current scientific knowledge

This IRM plan is based on the high-dose/refuge strategy. The strategy consists in planting *Bt* maize that produces sufficiently high concentrations of the insecticidal Cry protein so that even partially resistant target pest individuals do not survive. A non-*Bt* refuge is planted nearby providing a safe and large enough habitat for susceptible target pest individuals, so resistant insects emerging from the *Bt* maize field are likely to mate with susceptible insects from the refuge producing a heterozygous progeny that is phenotypically susceptible to *Bt*-maize (Head and Greenplate, 2012). The value of this approach has been demonstrated through mathematical modelling and field experiments (Ives and Andow, 2002; Shelton *et al*., 2000) and is considered an effective tool in delaying the development of resistance in *Bt* crops (MacIntosh, 2009; Huang *et al*., 2011; Head and Greenplate, 2012).

Three key assumptions underlie the high-dose/refuge strategy: the plant must express the toxin at sufficient levels so that resistance is functionally recessive, resistant insects must mate randomly with susceptible individuals surviving in the refuge, and resistance alleles must be rare (Andow, 2008).

[2] http://ec.europa.eu/food/plant/gmo/reports_studies/index_en.htm (Accessed July, 2012)

The *Bt* maize crops included in this IRM plan, MON 810, Bt11 and TC1507 express the *Bt* protein at high dose. Current scientific knowledge suggests that the frequency of resistance alleles in populations of ECB and MCB in Europe is low and that these alleles are recessive (Bourguet *et al.*, 2003; Gaspers, 2009). Knowledge on ECB and MCB biology and previous experience of cultivation of *Bt* maize in Spain and implementation of IRM measures following the high-dose/refuge strategy suggest that the high-dose refuge strategy is a suitable tool for delaying the development of resistance in ECB and MCB in Europe.

For ECB, many studies have been conducted to determine the genetic diversity and baseline susceptibility of ECB populations to Cry1Ab and Cry1F. The results showed that there is a low genetic differentiation of ECB populations in Europe and no geographic clusters of populations have been detected (Gonzalez-Nuñez *et al*., 2000; Chaufaux, 2001; Farinós *et al.*, 2004; Gaspers, 2009; Gaspers *et al*., 2011). This was also confirmed by analysis conducted with ECB in Europe by Saeglitz (2006). Baseline susceptibility of ECB in populations collected from different EU countries showed some variability, but no consistent pattern emerging, suggesting that there is an intra-species variability in susceptibility to Cry1Ab and Cry1F (Gaspers, 2009 and Gaspers *et al*., 2011).

For MCB, studies have also been conducted to determine the genetic diversity and baseline susceptibility to Cry1Ab and Cry1F (Gonzalez-Nuñez *et al*., 2000; De La Poza et al., 2008; Farinós *et al.*, 2012). The results showed that population genetics of MCB collected in populations in Spain and southwest France were closer than populations from Italy, Greece, and Turkey (De la Poza *et al.,* 2008), suggesting a small genetic differentiation between West Mediterranean and East Mediterranean populations. However, no significant differences in the susceptibility to Cry1F and Cry1Ab were found when comparing MCB populations from these two areas (Farinós *et al.*, 2011 and 2012).

As discussed in Head and Greenplate (2012) there are a number of factors that can influence the development of resistance in insect pests. Apart from the characteristics of the product and the genetics of resistance, the pest ecology (such as movement and mating and the number of generations per year) can influence the development of resistance. In Europe, ECB completes one or two generations per year depending on latitude, generally with one generation in the North of Europe and two in the South (Farinós *et al.*, 2004). Whereas MCB completes a variable number of generations per year depending on latitude, ranging from two in southern France to up to four in Morocco (Farinós *et al.*, 2012). The mating behaviour and movement of these species has also been studied (Showers *et al*. 2001, Hunt et al., 2001; Tate *et al.,* 2006; Eizaguirre *et al*., 2004 and 2006; Reardon and Sappington, 2007).

In summary, there is a lot of information on the baseline susceptibility of ECB and MCB populations to Cry1Ab and Cry1F in Europe, the genetic diversity within populations of these species and their ecology. The scientific findings suggest that the implementation of a high-dose/refuge strategy is a suitable tool to delay the onset of resistance to *Bt* maize in ECB and MCB in Europe.

4 Characteristics of the IRM plan

The goal of the IRM plan is to detect resistance in advance of population resistance to allow adequate time for the confirmation and characterization of resistance, and to take steps to reduce the likelihood or extent of product failure. The plan has been designed to be effective, balanced and practical for the growers of *Bt* maize.

4.1 Effective

Based on current knowledge of pest biology and insect resistance, combined with information from simulation models incorporating highly generous safeguard margins, a science based IRM plan has been developed.

Recognising that available data may not be representative of all pest populations and that a degree of uncertainty exists, the present IRM plan incorporates generous safeguard margins to ensure that the IRM plan is precautionary. In particular, the added safeguard margins are manifested by a larger refuge than would be necessary in the EU on strictly technical grounds. A comparable refuge strategy has been used in the USA where *Bt* maize has been grown widely on a commercial scale since 1996. Despite extensive monitoring efforts over the past 16 years, there has been no report of development of ECB resistance to *Bt* maize in the USA (Siegfried *et al.*, 2007; Crespo *et al*., 2009; Head and Greeplate, 2012). The effectiveness of the IRM plan will be reviewed regularly, taking into account the results of resistance monitoring to incorporate any new scientific developments relevant to the IRM plan.

4.2 Balanced and practical

It is important that all stakeholders of *Bt* maize technology adopt and implement the elements of the IRM plan. Seed companies have experience in cooperating with regulatory agencies, providing grower education, implementing product stewardship and working with experts on resistance management initiatives. However, farming practices are also critical to the success of the IRM plan. This highlights the importance of the decision-making of individual growers in the implementation of the IRM plan, in particular the refuge strategy. These important factors have been taken into consideration whilst developing the IRM plan, in particular the recommendations for implementation of a refuge, which have been carefully designed to be pragmatic, clear and consistent across relevant regions as well as provide a degree of flexibility where necessary according to variable cropping systems.

The refuge requirement is part of the IRM plan and is designed to delay the potential development of resistance by target pests to *Bt* maize. This is a precautionary measure to reduce the selective pressure on local populations of target pests. Details on refuge size, location, configuration and a tested process for investigating unexpected damage are provided in the IRM plan. The practices described in this plan balance a grower’s opportunity to benefit from *Bt* maize in the short term with the longer-term objective of preserving the efficacy of *Bt* maize. All developers subscribing to the present IRM plan are committed to provide farmers with the necessary guidance, technical support and advice on best practices for growing *Bt* maize.

5 Elements of the IRM plan

The IRM plan is comprised of the following elements:

- Maintaining an adequate level of non-*Bt* maize refuge in the vicinity of *Bt* maize to support a sufficient local population of susceptible target pests.
- Baseline susceptibility data for ECB and MCB have been established for Cry1Ab and Cry1F.
- Monitoring for any potential development of resistance.
- Remedial action plan in case of any confirmed development of resistance.
- Programme of grower education for greater awareness of *Bt* maize cultivation and proper stewardship

The first four elements are elaborated below. Details about grower education can be found in Section 6.

5.1 Refuge

Currently, it is widely accepted that resistance to single insecticidal trait *Bt* crops is rare and genetically recessive (Head and Greenplate, 2012). This has led to the development of IRM plans using a high-dose/refuge strategy based on the following assumptions:

- *Bt* maize that produces sufficiently high concentrations of the insecticidal Cry protein so that even partially resistant target pest individuals do not survive
- Resistance alleles typically are partially or fully recessive and rare so there will be few homozygous survivors
- Refuges are set up so that resistant homozygotes will mate randomly with susceptible individuals.

In summary, the purpose of the refuge is to maintain high numbers of susceptible homozygotes that will breed with the few surviving heterozygotes as well as with the rare resistant homozygotes, thereby delaying the evolution of resistance.

The effectiveness of a refuge is dependent on biological, genetic, behavioural and social or cultural factors. Therefore, the refuge strategy described below takes into account EU target pests, agronomic conditions and cultural practices. Moreover, it draws from experience gained through several years of implementing refuge strategies in countries where *Bt* maize is routinely cultivated. The result is a refuge strategy that incorporates generous safeguard margins and will delay or avoid resistance of target pests to *Bt* maize without compromising grower accessibility to *Bt* maize or grower ability to implement refuge requirements.

5.1.1 Refuge size

An appropriate level of refuge should be determined based on a comparative analysis of refuge strategies and maize-growing conditions in countries where *Bt* maize is regularly cultivated. The minimum proportion of non-*Bt* refuge implemented in other countries ranges from 10% to 20%. Such refuge sizes are considered to contain generous safeguard margins taking into consideration the local growing conditions.

For the purpose of the present IRM plan for the EU, a grower is defined as the individual responsible for managing and taking planting decisions on one farm or a group of farms. Growers planting more than 5 hectares (ha) of *Bt* maize would be required to plant a non-*Bt* maize refuge whereas growers planting less than 5 ha of *Bt* maize would not. This 5 ha threshold relates to the total amount of *Bt* maize, within or among fields, planted by one grower and is independent of the size of the individual fields or the total land area managed by this grower.

The EFSA scientific opinion on the annual monitoring reports by Monsanto on the cultivation of GM maize MON 810 in 2009 and 2010 concluded that the 5 ha threshold proposed is reasonable and conservative, given current scientific knowledge on the mating and movement behaviour of ECB and MCB in maize (EFSA, 2011 and 2012). However, the EFSA opinion highlighted that there could be areas of high *Bt* maize adoption where most of the maize fields could be 5 ha or less. Therefore, this IRM plan takes into account that in those areas the recommendations for refuge plantings will be reassessed to achieve the requested refuge levels.

5.1.2 Refuge configuration and placement

Refuge maize can be located near, adjacent to or within *Bt* maize fields. Refuges within a *Bt* maize field can be planted as a block, perimeter border or strips (see example in Appendix 1). Growers should also ensure that the refuge maize and the *Bt* maize share similar growth and development characteristics.

Growers should plant the refuge within 750 meters of their *Bt* maize field(s) although lesser distances are preferred. The objective of this distance requirement is to maintain a high probability of pest immigration into *Bt* maize, and consequently, a high probability that any rare individuals surviving on *Bt* maize will mate with susceptible individuals from the refuge. The scientific basis for this distance requirement is outlined in the work of Showers *et al.* 2001; Hunt *et al.* 2001 and Eizaguirre *et al.*, 2004 and 2009. This distance is also consistent with refuge strategies practiced in other countries.

Guidelines for planting a refuge will be clearly communicated in the product use guide that accompanies *Bt* maize.

5.1.3 Refuge management

Refuge zones should be managed in the same way as the *Bt* crop areas, where possible. Growers are encouraged to monitor their maize crop. Control of pest populations in non-*Bt* refuge maize should only be applied when the level of pest damage reaches economic importance. Where necessary, insecticides should be used according to their label recommendations. Microbial *Bt* sprays are the only class of insecticide that must not be used in refuge maize.

5.2 Resistance evolution in lepidopteran target pests

5.2.1 Introduction

Case Specific Monitoring (CSM) is hypothesis driven and should be carried out in order to confirm assumptions made in the Environmental Risk Assessment (ERA) and to further inform the ERA.

Resistance to chemical insecticides is known to evolve in insect pests therefore the potential evolution of insect resistance to Cry proteins expressed in *Bt* crops is considered to be a potential agronomic concern. The evolution of resistance in lepidopteran target pests has to be monitored in order to detect potential changes in resistance levels in pest populations.

The aim of the CSM is to detect resistance far enough in advance of building up of population resistance to allow adequate time for the confirmation and characterisation of resistance, and to take steps to reduce the likelihood or extent of product failure. A timeframe of two to two and a half years between initial resistance detection and implementation of an appropriate remediation plan is considered adequate.

CSM of insect resistance shall be undertaken for as long as cultivation of *Bt* maize continues. The effectiveness of the CSM will be reviewed regularly alongside the Insect Resistance Management (IRM) plan and shall incorporate other available relevant evidence and any new scientific developments into an updated PMEM and/or IRM plan as necessary.

5.2.2 Monitoring strategy

The authorisation holder will monitor the target organisms *Ostrinia nubilalis* (European Corn Borer - ECB) and *Sesamia nonagrioides* (Mediterranean Corn Borer - MCB) for changes in susceptibility to the expressed Cry protein. Baseline susceptibility measurements for the target organisms have already been established[3].

The sampling strategy for monitoring insect susceptibility in a given geographical area will be dependent on the ecology of the pests (based on current knowledge) and proportionate [representative of the cropping area] to the adoption levels of *Bt* maize. Sampling will take place in areas with high adoption of *Bt* maize and where the pest is present, as detailed in Table 3. Target pests within *Bt* fields will be constantly exposed to the Cry protein, therefore, reduction in the susceptibility of target pest individuals is likely to first appear in *Bt* fields. Potential resistant individuals will randomly mate with susceptible individuals in the same area and spread the resistance allele within the population. Measuring the susceptibility of a sample of individuals of that population will be a measure for the resistance allele frequency in that population. By comparing with the baseline data, resistance evolution is best measured.

Considering that the recommended size of the non-*Bt* maize refuge in the EU is 20%, the approach that will be followed for sampling is outlined in Table 3. *Bt* maize adoption levels could vary from year to year. The sampling methodology should be adapted to these variations.

Table 3. Sampling approach for insect resistance monitoring of ECB and MCB based on their ecology and levels of *Bt* maize adoption

***Bt* maize adoption rate per area(1)**	**Generations of ECB and MCB**	
	Univoltine	**Multivoltine**
< 20%	No sampling	No sampling
20-80%(2)	Monitoring every two years	Monitoring every two years
>=80%	Monitoring every two years	Monitoring every year

(1) A maize area is defined as a geographical zone where maize is typically grown following similar agronomic practices and isolated from other maize areas by barriers that might impair an easy exchange of target pests between those areas. The *Bt* maize adoption rate is expressed as a fraction of total maize cultivation in the same area, which is based on the data available for this area.

(2) Where the adoption rate of *Bt* maize remains below 80% it is likely that sufficiently large areas of non *Bt* maize will remain providing mosaics of both *Bt* and non *Bt* maize at regional scales.

Once an area has been identified for sampling, three independent samples of the relevant target pests shall be collected before the end of the cultivation season. Sample site selection within an area shall be determined by the target pest population which must be large enough to provide sufficient numbers of healthy individuals for collection. In addition, target pest collections should be made in non-*Bt* fields within the dispersal range of the insects coming from the nearest *Bt* maize field. The precise collection locations will be varied from year to year to provide thorough coverage sampling seasons. Based on the available information, these locations will be chosen in hotspots, *i.e.*, locations with the highest adoption of *Bt* maize and where the target pests are more likely to be multivoltine.

[3] Insert references.

5.2.3 Monitoring protocol

Sampling in areas will aim to detect a resistance allele frequency between 1-5%. Susceptibility tests will be performed based on a discriminating dose (Marcon et al 2000) with F1 progeny larvae from field collected individuals. Approximately 200 larvae, 200 adults, 100 mated females or 100 egg masses will be collected per population. A minimum population size of 60 larvae or 60 adults or 25 mated females or 25 egg masses will be considered a valid sample for testing.

Alternatively, F2 screening can be used. Susceptibility tests will be based on bioassays performed with a discriminating dose (Marcon et al. 2000[4]) with F1 progeny larvae from field collected individuals. Where F2 screening is used mated females will be considered to be the preferred stage for initiating an F2 screen. From the F1 progeny of the field collected adults single female lines will be reared and sub-mating within each of these lines will produce F2 offspring on which bioassays will be conducted.

The sampling will include collecting points close to *Bt* maize fields for which farmers have reported an unusual presence of damaged maize plants and of surviving target pests as part of the general surveillance outlined in Section 4.

5.2.4 Remedial plan in case of *Bt* maize failure to protect against target pests

The authorisation holder will ensure that information, documentation, technical guides are provided to seed companies, agronomic advisers, farmers and other users, pointing to the need to report unexpected and/or adverse effects to the authorisation holder.

5.2.4.1 Procedures for unexpected damage

The following procedures will be followed where there are reports of substantial damage:

a) The authorisation holder will request distributors to instruct purchasers of *Bt* maize seed to report unexpected levels of damage caused by target pests as and when they occur directly to the seed company.
b) If the seed company is a licensee for the *Bt* trait, it will transmit this information to the authorisation holder.
c) The authorisation holder will investigate the cause of the reported unexpected damage, using available methods to confirm that the damaged plants express Cry protein, the damage resulted from a target pest and the damage is unexpected.
d) Insects will be collected by the authorisation holder for the purpose of further evaluation

5.2.4.2 Steps to confirm resistance

a) If unexpected damage occurs the collected insects will be tested in a laboratory following specific guidelines with the aim being to:
   i. confirm field resistance;
   ii. confirm resistance is heritable;
   iii. use crosses to determine the nature of resistance (i.e. recessive or dominant, and level of functional dominance);
   iv. estimate r-allele frequency in the original population;
   v. determine whether the r-allele frequency is increasing by analysing field collections in subsequent years sampled from the same site where the resistant allele(s) was originally collected;

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[4] Marcon et al (2000) Development of diagnostic concentrations for monitoring *Bacillus thuringiensis* resistance in European Corn Borer (Lepidoptera, Crambidae). Journal of Economic Entomology. 93(3). 925-930

vi. determine the geographic distribution of the r-allele by analysing field collections in subsequent years from sites surrounding the site where the resistant allele(s) was originally collected;

b) Both of the following conditions must be met to confirm resistance: the collected insects or their progeny must have an MIC_{50} (minimum inhibitory concentration) that exceeds the upper 95% confidence interval of the historical (susceptible) mean MIC_{50} for the appropriate *Bt* protein and the collected insects or their progeny must achieve > 30% survival and > 25% leaf area damage in a 5-day bioassay under laboratory conditions using the appropriate protein-positive leaf tissue.

If resistance is confirmed**,** the authorisation holder will inform the European Commission and other relevant national Authorities according to the relevant legislation and take appropriate measures as described below.

6 Implementation (Grower education)

An extensive grower education programme is essential for the successful implementation of the IRM plan. Growers should have a clear understanding of the importance of IRM to preserve the long-term efficacy of the *Bt* technology and realise that their participation in this IRM stewardship programme is vital to prolonging the success and benefits of *Bt* maize. Each of the seed companies participating in this IRM plan is committed to continuing with their ongoing comprehensive education programmes.

A technical user guide will provide each purchaser of *Bt* maize with latest information on the recommendations for the IRM plan, *Bt* technology, the approval status of various *Bt* maize hybrids in the relevant country and contact details of the responsible seed provider (technology provider, licensee). The user guide will request growers to implement the required IRM measures such as recording where *Bt* maize is planted, planting a non-*Bt* maize refuge and monitoring product performance.

In addition, the IRM plan will be communicated using a combination of the following means:

- Slide and video presentations to growers and distributors, co-ops, seed dealers and distributors.
- Information via company and relevant country specific associations as well as agricultural extension services web sites.
- Newsletters.
- Country specific hotlines.
- Relevant competent authorities.

An example of the IRM guidance given to costumers in Spain is provided in Appendix 1 and will be adapted to the conditions of the local market.

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Appendix 1: Example of grower information material

✓ Es obligatorio, de acuerdo con la autorización europea y se especifica en las condiciones de empleo que el agricultor asume al adquirir la semilla (Etiquetas y Guías en sacos)

Ejemplo de la información recogida en las Guías Técnicas

Ejemplo de la información recogida en los sacos

La siembra de maíz Bt requiere la implantación de un programa de manejo de la resistencia de Insectos (IRM) que consiste en la siembra de un refugio de maíz no Bt para reducir el riesgo de desarrollo de resistencias antes de que éstas puedan producirse. Para más información, consultar la guía Técnica de YieldGard.

anove

3

¿Qué consecuencias tendría el desarrollo de poblaciones de taladro resistentes?

- ✓ **El maíz Bt dejaría de ser efectivo** contra los taladros.
- ✓ **Todos los agricultores** de esa zona **perderían** el acceso a la tecnología.
- ✓ Se crearía una **imagen negativa** de los agricultores españoles por descuidar el respeto a las Buenas Prácticas.

anove

4

¿Cómo debe sembrarse el refugio ?

- ✓ Obligatorio cuando se siembren más de 5 ha de maíz Bt, aunque éstas estén distribuidas en varias parcelas.
- ✓ Un tamaño de al menos un 20% de la superficie dedicada a maíz.
- ✓ Sembrado lo más cerca posible al campo con maíz Bt (distancia inferior a 750 m).
- ✓ Empleando una variedad convencional de ciclo y fecha de siembra similar.
- ✓ No sirve la parcela del vecino, ya que una parcela con maíz convencional de otra finca puede ser el refugio del propietario de dicha finca.
- ✓ Se puede tratar contra taladro, siempre que no se utilicen preparados de *Bacillus thuringiensis* (Bt)

Diferentes opciones son posibles

6

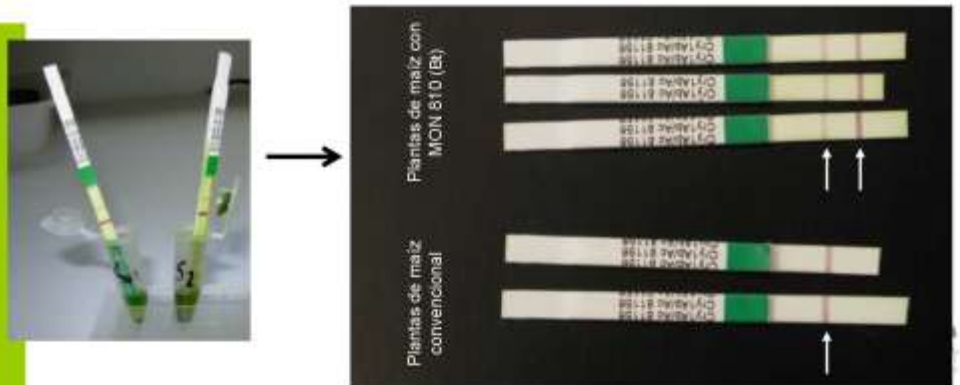
Las empresas que comercializan maíz Bt se toman muy en serio el empleo correcto de la tecnología

- ✓ Asegurando que los usuarios reciban información completa sobre Refugios y Buenas Prácticas, para cumplir con la legislación vigente.
- ✓ Recogiendo larvas de taladro cada año, que son analizadas en laboratorio para comprobar que siguen siendo sensibles a Bt.
- ✓ Evaluando a través de encuestas a agricultores el comportamiento de Bt en el campo y el grado de cumplimiento en la siembra de refugios.

7

ANOVE y las empresas comercializadoras ofrecen sus servicios para información, o comprobación en campo

✓ Comprobación de refugios en campo mediante análisis de hoja



8

Recuerde que:

Si el taladro se vuelve resistente, perdemos todos.

Muchas Gracias por su Colaboración

9

Appendix 2: Standard Operating procedures for eggs and larvae sampling