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

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CropLife Europe ERA Expert Group

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Summary

Insect resistance has been a challenge regardless of insect control approach (e.g., chemical/biological insecticides, conventional genes, transgenic approach). With the commercial cultivation of *Bt* crops, the possible development of insect resistance that could deprive growers of the benefits of *Bt* crops and *Bt* microbial preparations must be considered. Therefore, insect resistance management (IRM) plans have been implemented to delay resistance development. In the EU, with the introduction of *Bt* maize cultivation in 1998 in Spain, research programmes were initiated to establish susceptibility baselines for the targeted pests and subsequently monitor the potential development of insect resistance. In 2001, with the introduction of Directive 2001/18/EC (EC 2001), IRM plans became mandatory. Given the fact that *Bt* maize from different transformation events targeting the same insect pests were commercialized in the EU at that time (*Bt*176 and MON 810) and other transformation events were under review for approval (1507, *Bt*11), technology providers united efforts and proposed a common IRM plan. The purpose of this harmonized plan was to develop and use common methodology to manage and monitor for potential resistance to Cry1Ab and Cry1F endotoxins in European corn borer (ECB; *Ostrinia nubilalis*) and Mediterranean corn stalk borer (MCB; *Sesamia nonagrioides*) following the cultivation of these *Bt* maize varieties. The plan was implemented in 2003 (Alcalde *et al.,* 2007) and has been in place since then. Despite 19 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 is 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 harmonised plan is to keep it updated based on new learnings and scientific information. Since the first implementation of the harmonized IRM plan, there have been updates in the regulatory framework, a large amount of additional data generated in the scientific literature, and experience gained from IRM plans established in other regions. Therefore, the IRM plan has been updated incorporating the learnings from the additional available information.

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

Abbreviations, terms and definitions

ABSTC	Agricultural Biotechnology Stewardship Technical Committee
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
CONABIA	Comisión Nacional Asesora de Biotecnología Agropecuaria
Cry protein	Crystal protein derived from *Bt*
CSM	Case-specific monitoring
DC	Diagnostic concentration: a toxin (*Bt* protein) concentration that discriminates between resistant and susceptible insects in a bioassay based on a measurement of insect survival or development
ECB	European corn borer
EFSA	European Food Safety Authority
Endotoxin	Toxic molecule associated with the outer membrane and cell wall of bacteria
EPA	Environmental Protection Agency
ERA	Environmental risk assessment
EU	European Union
GM	Genetically modified
Grower	Individual responsible for seed purchasing, planting and on-farm stewardship
IPM	Integrated pest management
IRM	Insect resistance management
MCB	Mediterranean corn stalk borer
Field resistance	Field resistance is defined as a genetically-mediated ability of a target pest to survive on a commercial line(s) of *Bt* maize (single insecticidal trait) under field (or near field such 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* maize. Fitness costs (e.g. delayed development, reduced competitiveness, or fecundity) may be associated with the resistance.
INTA	Instituto Nacional de Tecnología Agropecuaria
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 under laboratory conditions. Such resistance may be observed as an increase in population mortality or developmental response, 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 resistance on *Bt* maize plants in the field.

Population resistance	Population resistance occurs when a large portion of a target pest population is field-resistant and causes the *Bt* maize to fail to confer economic control of the population.
PMEM	Post-market environmental monitoring
SCB	Sugarcane borer
USA	United States of America

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 have limited ability to 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 (Fitt and Wilson 2000).

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 additional management tool to control crop damage and losses due to insect pests. The use of insect protected crops provide important benefits to growers, society, and the environment (Brookes and Barfoot 2009b; MacIntosh 2009; Brookes and Barfoot 2009a; Gómez-Barbero *et al.,* 2008; Kendra and Dyer 2007).

Maize plants expressing *Bt* proteins for pest control were first registered for commercial use (deregulated) in the United States of America (USA) in 1996. Currently, genetically modified (GM) maize containing an insect protection trait (as such or in combination with herbicide tolerance) is one of the most widely planted GM crops, as can be found in the annual reports of ISAAA (http://www.isaaa.org).

Insect resistance has been a challenge before development of Bt crops, as it can develop with use of conventional pesticides or conventional genes (Smith 2004). 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 of insect pests to pesticides is a well known phenomena and a number of confirmed cases of field resistance to *Bt* crops have also been reported over the past decade (Dhurua and Gujar 2011; Farias *et al.,* 2014; Gassmann *et al.,* 2011; Storer *et al.,* 2010; Van Rensburg 2007).

Biotechnology companies have been working with academic experts, regulators and growers to design and implement proactive insect resistance management (IRM) 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, either in the European Union (EU) or on a global scale.

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 events 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 (1507, *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 manage and monitor for the potential development of resistance to Cry1Ab and Cry1F endotoxins in ECB and MCB following the cultivation of these *Bt* maize events. The plan was implemented in 2003 (Alcalde *et al.,* 2007) and has been in place ever since. Despite more than 15 years of use of *Bt* maize in the EU and high adoption rates of the technology in some areas, no decrease in the susceptibility of either ECB or MCB to Cry1Ab has been detected (Castaňera *et al.,* 2016). This suggests that the harmonized

IRM plan is effective for Cry1Ab. However, one of the elements described in the plan is to keep it updated in view of the gathered experience in Europe and other regions, and new scientific information. Since the implementation of the first harmonized IRM plan, there have been some updates in the regulatory framework. Considering all this information, the IRM plan has been updated in 2017.

This document describes the updated IRM plan including the key guidance elements and the rationale behind the recommendations. The current updated IRM plan is in line with the recommendations and guidance provided by the current regulatory framework. The goal of the IRM plan is to delay the development of insect resistance, detect changes in pest susceptibility, and if necessary confirm and characterize alleged cases of reported resistance and take appropriate mitigation steps. The plan has been designed to be effective and balanced while remaining practical for the growers adopting *Bt* maize.

2 Scope of the plan

The transformation events currently included in the proposal are presented in Table 1 and are further referred to as *Bt* maize in this document.

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

Transformation event	OECD unique identifier	Protein	Notifier
Bt11	SYN-BT∅11-1	Cry1Ab	Syngenta
MON 810	MON-∅∅81∅-6	Cry1Ab	Bayer
1507	DAS-∅15∅7-1	Cry1F	Pioneer/DAS member of Corteva Agriscience group

The main insects targeted by the plan are ECB and MCB, 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 EU

The updated IRM plan proposed by CropLife Europe takes into account the recommendations and guidance provided by the current regulatory framework.

Directive 2001/18/EC (EC 2001) was the first Directive to establish that notifiers should develop and submit a monitoring plan together with the notification for placing on the market of a GM crop. The design of the post-market environmental monitoring (PMEM) plan was outlined in Annex VII of this Directive. The objectives of the monitoring plan were: (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 (EC 2004) 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) thereby providing recommendations on the approach for conducting PMEM.

3.2 Practical experience from IRM plans implemented around the world

IRM is multifaceted, there are multiple factors that can contribute to evolution of insect resistance.

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 grower 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 management and monitoring of insect resistance development to *Bt* crops as a condition of registration (USEPA 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 refuge requirements, grower agreements and resistance monitoring programs for Cry1Ab and Cry1F *Bt* maize (Siegfried *et al.,* 2007b). To date, despite the high level of adoption of *Bt* maize in the USA during more than two decades, there are no reports of field-evolved resistance in populations of ECB to Cry1Ab or Cry1F (Head and Greenplate 2012; Siegfried *et al.,* 2007b; Tabashnik *et al.,* 2003; Tabashnik *et al.,* 2009).

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 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 primary local target pest, the sugarcane borer (SCB), 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

[1]https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/introduction-biotechnology-regulation-pesticides (Accessed September 2023).

Biotecnología Agropecuaria (CONABIA) and implemented. To date, there are no reports of field resistance of SCB to Cry1Ab. Resistance of SCB to Cry1F has been reported from a single small isolated area called San Luis where, in addition to other environmental factors, refuge implementation was subsequently found to be very low. Mitigation practices were put in place and the resistance has not been observed outside this area.

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 varies depending on the biology and ecology of the target pests in that country. In South Africa, there have been confirmed resistance cases of *Busseola fusca* developing resistance to *Bt* maize in the field, however, these cases also have been linked to poor implementation of IRM practices among customers; further customer education efforts and monitoring efforts have slowed the spread of resistance (Kruger *et al.,* 2012; Van Rensburg 2007). No cases of resistance in ECB and MCB have been reported worldwide for Bt maize.

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 improved the understanding of baseline susceptibility of the target pest populations in different EU countries, and on susceptibility levels after continuous exposure to *Bt* maize and on the ecology of the pests (See Section 3.4 for a summary). In addition, since the only *Bt* maize currently cultivated in the EU is MON 810, Bayer, in compliance with current regulatory requirements, has submitted annual monitoring reports to the European Commission since 2005[2]. These reports provided information on the findings of the implementation 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). Since 2011, EFSA has published scientific opinions in which recommendations to improve the methodology for MON 810 PMEM are made (EFSA 2011b, 2012, 2013, 2014, 2015, 2016, 2017). These scientific opinions and the recommendations therein have changed over time, and the outcome of the most recent scientific opinions have been taken into consideration while updating this IRM plan, reflecting the experience gained with MON 810 PMEM.

One of the key conclusions of all this research is that no shifts in susceptibility have been observed for field populations of ECB or MCB after more than 15 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 of 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 (Head and Greenplate 2012; Huang *et al.,* 2011; MacIntosh 2009).

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

[2] https://ec.europa.eu/food/plant/gmo/reports_studies_en (Accessed September 2023)

with susceptible individuals surviving in the refuge, and resistance alleles must be rare (Andow 2008).

The *Bt* maize crops included in this IRM plan, MON 810, Bt11 and 1507 express the *Bt* protein at high dose for the targeted pests, ECB and MCB. 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 low genetic differentiation of ECB populations in Europe and no geographic clusters of populations have been detected (Chafaux *et al.,* 2001; Farinós *et al.,* 2004; Gaspers 2009; Gaspers *et al.,* 2011; Gonzalez-Nuñez *et al.,* 2000). This was also confirmed by analysis conducted with ECB in Europe by Saeglitz (Saeglitz *et al.,* 2006). Baseline susceptibility of ECB in populations collected from different EU countries showed some variability, but no consistent pattern emerged, suggesting that there is limited intra-species variability in susceptibility to Cry1Ab and Cry1F (Gaspers 2009; Gaspers *et al.,* 2011).

For MCB, studies have also been conducted to determine the genetic diversity and baseline susceptibility to Cry1Ab and Cry1F (De la Poza *et al.,* 2008; Farinos *et al.,* 2012; Gonzalez-Nuñez *et al.,* 2000). 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 (Farinos *et al.,* 2011; Farinos *et al.,* 2012).

As discussed in Head and Greenplate (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). MCB completes a variable number of generations per year depending on latitude, ranging from two in southern France to up to four in Morocco (Farinos *et al.,* 2012). The mating behaviour and movement of these species have also been studied (Eizaguirre *et al.,* 2006; Eizaguirre *et al.,* 2004; Hunt *et al.,* 2001; Reardon and Sappington 2007; Showers *et al.,* 2001; Tate *et al.,* 2006).

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 delay resistance development and detect changes in pest susceptibility, if and when they occur, so that they can be characterized and appropriate management steps can be taken to maintain product efficacy. The plan has been designed to be effective and balanced while remaining practical for the growers adopting *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.,* 2007a; Siegfried and Spencer 2002). The effectiveness of the IRM plan will be reviewed regularly in order 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. Agricultural technology providers have experience in cooperating with regulatory agencies, providing grower education, implementing product stewardship and working with scientific experts on resistance management initiatives. However, farming practices are 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 in 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 growers 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:

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

The abovementioned elements are elaborated below.

5.1 Refuge

Currently, it is widely accepted that resistance to *Bt* crops with single insecticidal traits is rare and genetically recessive (Head and Greenplate 2012). This encouraged 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, producing susceptible offspring, thereby delaying the evolution of resistance.

The effectiveness of a refuge is dependent on many factors. Therefore, the refuge strategy described below takes into account the biology, genetics and behaviour of EU target pests, agronomic conditions and cultural practices implemented by growers. 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 resistance development of target pests to *Bt* maize without compromising grower accessibility to *Bt* maize or grower ability to implement the outlined 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 for single trait products like MON 810, Bt11 and 1507 targeted at corn borers in other countries ranges from 5% 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 opinions on the annual monitoring reports by Bayer on the cultivation of GM maize MON 810 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. However, EFSA opinions on the cultivation applications for *Bt* maize state that in the case of a cluster of fields with an aggregate area greater than 5 ha of *Bt*-maize there should be refugia equivalent to 20% of this aggregate area, irrespective of individual field and farm size. However, it is not clear how such clustered fields should be defined and how the different parties, including growers in such a clustered field, could be granted access to this information. Moreover, in order to comply with such a requirement, clustered fields would need to be identified prior to planting and would therefore require compiling accurate planting intentions for all *Bt* maize growers, which is not reasonable for an activity that is driven by many factors out of the control of the growers (commodity prices, climate conditions, *etc*). Finally, it is not clear which of the growers with the fields located in a clustered area should purchase refuge seed. Based on these considerations, the recommendations for planting a structured refuge as laid down in this plan are focussed on individual fields or clustered fields from one grower that exceed the 5 ha threshold.

5.1.2 Refuge configuration and placement

Structured 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 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 smaller distances are preferred. The objective of this distance requirement is to maintain a high probability of pest immigration from the refuge 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 (Eizaguirre *et al.,* 2006; Eizaguirre *et al.,* 2004; Hunt *et al.,* 2001; Showers *et al.,* 2001). This distance is also consistent with structured 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 for the presence of the target pest(s). 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 the structured refuge maize.

5.2 Resistance monitoring 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 ERA and to further complement the ERA.

Resistance to chemical insecticides is known to evolve in insect pests. The potential development of insect resistance to Cry proteins expressed in *Bt* crops is considered to be a potential agronomic and economic concern. In order to detect potential changes in susceptibility levels in the target pest populations monitoring will be conducted.

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 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 responsible parties will monitor the target organisms ECB and MCB for changes in susceptibility to the expressed Cry protein. Baseline susceptibility measurements for the target organisms have already been established.

The sampling strategy for monitoring insect susceptibility in a given geographical area will depend 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. Since target pests within *Bt* fields are constantly exposed to the Cry protein, reduction in the susceptibility of target pest individuals is likely to first appear in these fields. Potential resistant individuals will randomly mate with susceptible individuals in the same area and spread the (recessive) resistance allele within the population. Measuring the susceptibility (by dose-response or diagnostic concentration assays) 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, the evolution of resistance can be assessed.

Considering that the recommended size of the non-*Bt* maize structured 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, when Bt adoption rate information is available, 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**
< 60%	No sampling	No sampling
60-80%(2)	Monitoring every two years	Monitoring every year
>=80%	Monitoring every year	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, 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 across 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.

5.2.3 Monitoring protocol

Susceptibility tests will be performed in initial years of product introduction as a concentration-response and then, once validated, a discriminating concentration (Marçon *et al.,* 2000) will be tested with F1 progeny larvae from field collected individuals. Approximately 1 000 larvae will be targeted for collection per population. However, from the Spanish experience gained during more than 10 years of MON 810 PMEM, there are clear indications that collection of 1 000 field larvae per sampling area to meet detection of 3% (recessive) resistance allele frequency as suggested by EFSA (EFSA 2016), will not always be feasible. Detailed information from independent, public available resources[3] demonstrates that the target pests' pressure, and consequently number of larvae, are reduced as a result of more than a decade of MON 810 cultivation and of the high performance of MON 810. In addition, when *Bt* maize expressing other events targeting the same pests will be cultivated, it is expected that target pest populations will continue to decrease. However, non-recessive resistance alleles will continue to be detected efficiently in laboratory assays even if sample sizes are much reduced in some years. Field monitoring of product performance and unexpected damage also will reveal any reduced susceptibility of the targeted pests in the field. Based on the above, it will not be possible to ensure the yearly collection of 1,000 larvae per sampling area. Therefore, flexibility should be granted, provided that the responsible parties can demonstrate to have undertaken the necessary steps to ensure appropriate larval collection. When multiple *Bt* maize events will be cultivated, a joint collection of 1,000 larvae per year could be pursued. The sampling will include collecting points close to *Bt* maize fields that have been identified with the information from companies selling *Bt* maize.

Another method that has been shown to be efficient in detecting resistance alleles at very low frequencies present in field populations is F2 screening and is therefore an appropriate tool to establish the initial inherent resistance allele frequency in target pest populations. The F2 screening is however not considered the preferred standard monitoring bioassay method because of the non-proportional use of laboratory resources.

5.3 Growers complaint system

Grower complaint systems provide a means for growers to report any unexpected effects when cultivating *Bt* maize in their field. Growers are first in line to detect a potential change in product performance which may be caused by reduced insect susceptibility. When a target pest control performance complaint is received by any company selling the *Bt* maize seeds, necessary steps will be taken in order to confirm decreased product performance in the *Bt* field. The procedure for when unexpected damage is reported is described in Section 5.4.

[3] Catalunya Research Institute, IRTA, 2014; https://www.ruralcat.net/c/document_library/get_file?uuid=52ce0d40-0c2f-42c8-ac9f3609cc656237&groupId=10136 (Accessed September 2023)

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

The responsible parties will ensure that information, documentation, trainings, and technical guides are provided to seed companies, agronomic advisers, growers and other stakeholders, pointing to the need to report unexpected and/or adverse effects to the responsible parties.

5.4.1 Procedures for unexpected damage

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

a) The responsible parties 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 biotechnology company.
b) If the biotechnology company is a licensee for the *Bt* trait, it will transmit this information to the authorisation holder.
c) The responsible parties 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 responsible parties for the purpose of further evaluation

5.4.2 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 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;
    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: 1) the collected insects or their progeny must exhibit a mortality or developmental response that exceeds the upper 95% confidence interval of the historical (susceptible) response for the appropriate *Bt* protein and 2) the collected insects or their progeny must achieve > 30% survival and > 25% leaf area damage in a bioassay under laboratory conditions using the appropriate protein-positive leaf tissue.

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

5.4.3 Remedial actions in case of Bt maize failure

Appropriate integrated pest management (IPM) options will be identified and implemented to minimize spread of the problem. The remedial actions should be implemented as soon as resistance is suspected.

5.5 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 biotech companies participating in this IRM plan is committed to execute 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* maize hybrids available in the relevant country together with 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 and reporting unexpected damage immediately, if any.

In addition, the IRM plan will be communicated using a combination of delivery mechanisms that may include 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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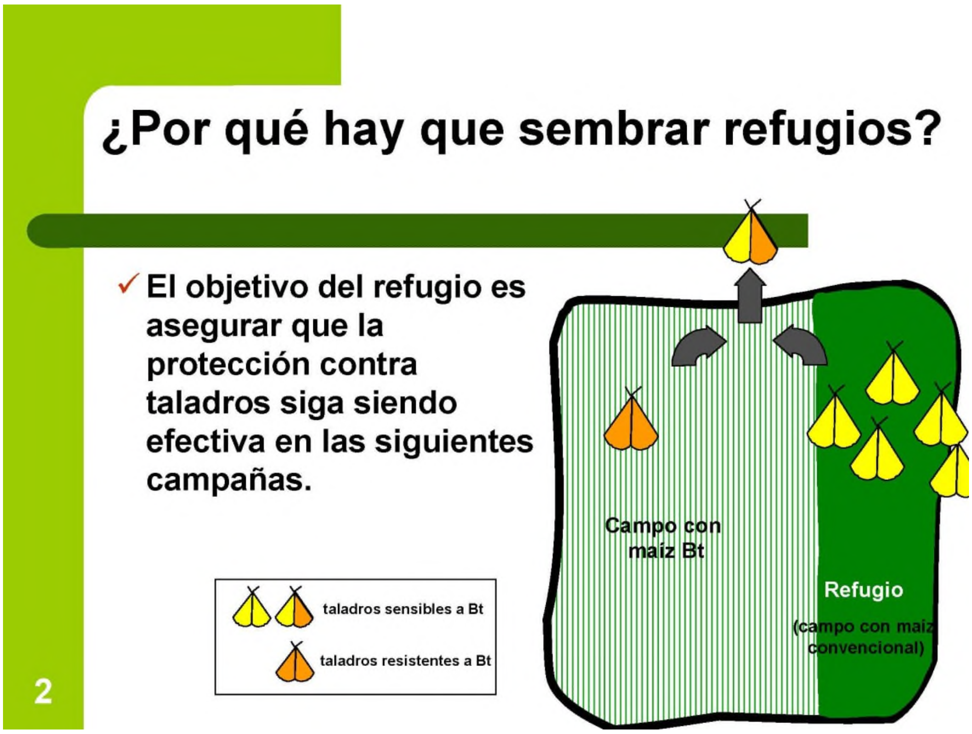
Appendix 1: Example of grower information material



- ✓ 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.

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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.

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¿Cómo debe sembrarse el refugio ?

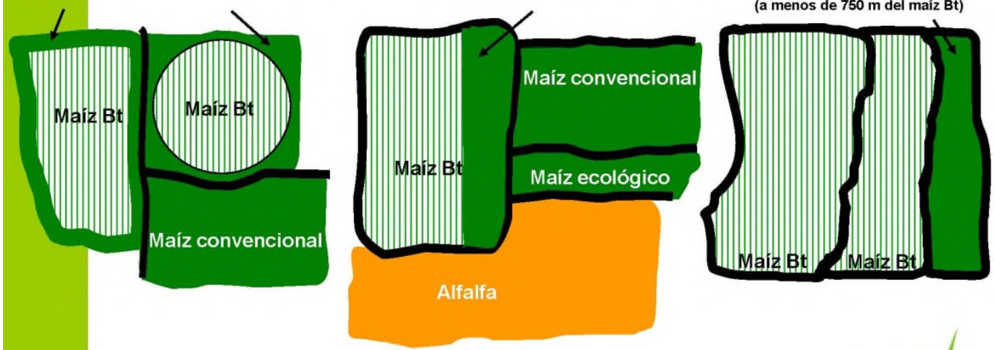
- ✓ **Obligatorio** cuando se siembren **más de 5 ha de maíz Bt,** aunque éstas estén distribuidas en varias parcelas.
- ✓ Un tamaño de al menos un **20%** de la superficie dedicada a maíz.
- ✓ Sembrado lo más cerca posible al campo con maíz Bt (distancia inferior a 750 m).
- ✓ Empleando una **variedad 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

Refugio sembrando las cabeceras o las esquinas del pivot con maíz convencional

Refugio sembrando maíz convencional en un bloque, que sirva de aislamiento a la parcela de maíz Bt

Refugio para agricultores con parcelas pequeñas, sembrando maíz convencional en una parcela completa.
(a menos de 750 m del maíz Bt)



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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.

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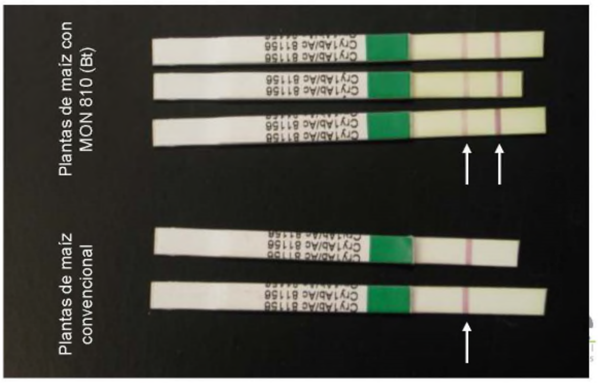
7

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

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9

Appendix 2: Standard Operating Procedure for the collection of the Mediterranean corn borer (MCB, *Sesamia nonagrioides*) and European corn borer (ECB, *Ostrinia nubilalis*).

Definition of a population and basic requirements:

Larvae are collected from areas with high adoption of Bt maize. Each area is made up by three sampling zones, each zone comprising at least three maize fields in the smallest possible surface. Larvae will be collected from non-Bt maize fields, near Bt crops. Taking into consideration a potentially reduced target pest pressure and consequently a potential low larvae abundance, approximately 1000 larvae of each species will be targeted for collection per area, about 350 larvae collected in each of the three sampling zones and, if possible, a minimum of 50 larvae per maize field.

Field selection process:

Before September, ask your local crew to help in the identification of fields having an optimal to maximum symptoms of MCB attack. The local crew is also responsible to ask the farm owner for access prior to the collection.

Best timing for collection of larvae is prior to the harvest.

Field collection procedure for MCB and ECB larvae:

Insure collection of:

• Field address, postal code, name of the area or bigger town the field is close to

• Date of collection

• Maize varieties in the field

• Each field insect has to be treated separately

General material:

- a big insulation box to store the collection boxes when they are complete

- water to moisten filter paper

- gloves and masks to avoid small scratches from maize leaves and maize pollen allergy

Material per person:

- a solid and sharp knife

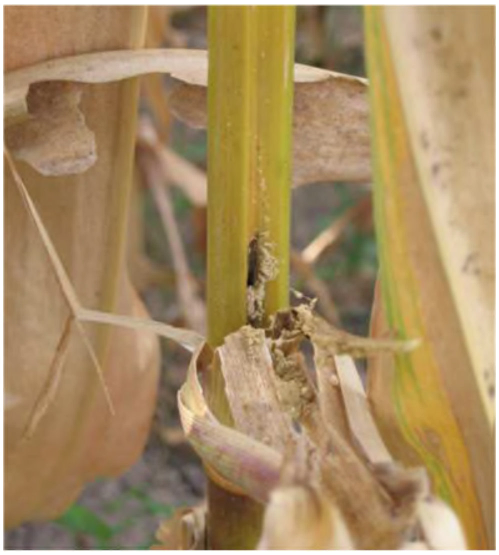
- soft and thin forceps

- collection boxes: they should be ventilated hard plastic boxes (crystal polystyrene) for storing larvae , filled with maize leaves and small talk pieces on moistened filter paper. The plastic should be hard enough to avoid that the larvae eat the plastic and escape (for instance, polyethylene plastic of Tupperware® can be bored by MCB and ECB, do not use it!). A wire mesh for ventilation should be used for the same reason.

Method:

- Look for plants with boreholes and fresh faeces, remove the leaves.

- Cut the stalk about 5 cm over the hole



- Split carefully the stalk with the knife following the line of the tunnel, using the knife as a lever, until you find the larva.

- Take one larvae of each species per plant solely to avoid collecting siblings and to maximize the genetic variation of the collection. If both the stalk and cob show boreholes, choose better the stalk, it is easier.

- Transfer larvae into the collection box (not more than 50 larvae of MCB and 75 larvae of ECB per box 21x16x5 cm; but the number will depend on the size of the box) containing stalk parts. Ensure that the box is not exposed to direct sunlight during the collection process and moisten the filter paper as necessary.

 MCB	 ECB

- If a collection box is filled up, moisten the filter paper and then place this box in the insulation box for transport. Assure to label the collection box appropriate if more than one population is collected and sent in one insulation box.

- Do not leave collection boxes with larvae within the car exposed to sunlight. The temperature inside rises so high that larvae could die.

Transportation procedure to send field collected larvae to the reference laboratory:

• Don't store the boxes for longer than 2 days before they were sent to labs.

• For the shipment of both species, the same ventilated boxes of hard plastic described before should be used. They should be closed tightly by means of a rubber band and/or adhesive tape. Thin stalk parts (avoiding thick and heavy pieces) and green leaves (which keep the suitable humidity inside the box) should be added for feeding and protection. Most of the larvae will bore the stalks and will be protected during the delivery. For ECB, replace maize stalk parts with corrugated cardboard if they are in diapause. If necessary, add soft crumpled paper to fill out the box, avoiding that the movement crush the larvae.

Diapausing larvae of ECB

• If you schedule a collection assure that the boxes will not be shipped later than **Wednesday!**

• A Friday collection should be avoided to do not put insects in difficult storage conditions. As well as a Friday parcel service delivery is not appropriate to avoid an over the weekend insect storage in transportation process.

• Do not expose the insect to hot condition or direct sunlight. Cool temperatures are optimal.

• If you arrange the shipment by yourself select an air plane rapid shipment service, and please use the company recommended by the service requestor. Please inform about the arranged shipment and the tracking number.