Appendix 6. EU Working Group on Insect Resistance Management: Harmonised Insect Resistance Management (IRM) Plan for Cultivation of *Bt* maize in the EU

EU WORKING GROUP ON
INSECT RESISTANCE MANAGEMENT

- Harmonised insect resistance management (IRM) plan for cultivation of Bt maize in the EU -

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Participating companies:
Monsanto Europe S.A.
Pioneer Hi-Bred International, Inc.
Syngenta Seeds S.A.S.

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Summary

Maize is an important crop in the European Union (EU) and corn borer infestations can result in considerable crop damage and yield loss. In Spain for example, the losses in maize production can be as high as 15% in areas of high corn borer pressure. The use of conventional insecticides is not practical against corn borers since chemical sprays cannot reach boring pest larvae whereas crops expressing a *Bacillus thuringiensis* protein for pest resistance, hereafter referred to as Bt crops, provide an in-built resistance to combat these pests. Since its introduction in the USA in 1995, Bt maize has proved to be a successful management tool to control crop damage and losses to insect pests. This technology has been widely adopted and its use extended to other countries.

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. Despite the fact that resistance to Bt in the field has not been detected to date, this concern has been addressed pro-actively in a number of countries by the implementation of insect resistance management plans to delay the potential development of pest resistance and to enable the timely detection of changes in pest susceptibility. Following the experiences gained in other countries and taking into account the latest scientific reports, an industry working group, the **EU Working Group on Insect Resistance Management** has developed a harmonised insect resistance management (IRM) plan specific for the EU.

The objectives of the Working Group are to assist in compliance with existing EU regulatory requirements with regard to the monitoring of Bt maize and to protect the Bt technology through good stewardship. The purpose of the IRM plan is to proactively avoid where possible, and in all cases delay the potential development of pest resistance to the Cry1Ab and Cry1F proteins as expressed in Bt maize. The Working Group is well aware that the key to success for the IRM plan is acceptance and adoption by European growers. Therefore it has been designed to be effective, balanced and practical. The harmonised IRM plan contains guidance on the following key elements:

- How to use the Bt technology: a comprehensive grower education programme will aid the grower in understanding the importance of insect resistance management to preserve the long-term efficacy of the Bt technology and in employing the required resistance management tool of implementing a generous 20 % refuge for Bt maize planting areas larger than 5 hectares
- Resistance monitoring: baseline susceptibility of European corn borer (*Ostrinia nubilalis*) and Mediterranean corn stalk borer (*Sesamia nonagrioides*) to Cry1Ab and Cry1F endotoxins of *B. thuringiensis* in the EU will be measured and monitoring techniques are described to detect changes relative to baseline susceptibility which could result in inadequate protection against *O. nubilalis* and *S. nonagrioides* in the field
- Potential development of resistance: confirmation of pest resistance and remedial action plan.

In summary, the harmonised IRM plan for the EU is designed to allow farmers to benefit from growing Bt maize while pro-actively avoiding where possible, and in all cases delaying the potential development of target pest resistance in the field.

1 INTRODUCTION

Bacillus thuringiensis (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 will provide important benefits to growers, society, and the environment (McGaughey and Whalon, 1992; Gasser and Fraley, 1989; Gould, 1988; Nester *et al.*, 2002).

Maize expressing a *Bacillus thuringiensis* protein for pest resistance, hereafter referred to as Bt maize, was first registered (deregulated) in the USA in 1995. Currently, it is the second most widely planted genetically modified (GM) crop, with approximately 5.9 million hectares commercially grown worldwide in 2001, mainly in the USA, Canada, Argentina, and South Africa. The introduction of Bt maize in the European Union (EU) is at an early stage. Small areas of Bt maize, approximately 20 - 25,000 ha or 5 % of the total Spanish maize growing area, have been planted each year in Spain since 1998. Token amounts have been planted in France in 2000 and in Germany in 2001 (ISAAA, 2001). A recent study (Brookes, 2002) demonstrated clear economic and environmental benefits for Spain in the Bt maize growing areas.

Although to date there is no literature report of ECB developing resistance to Bt microbial products in the field, the potential occurrence of resistance to Bt proteins arising from the use of Bt crops is generally accepted as a possibility. Therefore, in the interests of good product stewardship and to preserve the utility of the technology, the large-scale commercial introduction of Bt maize in various world areas has been accompanied by insect resistance management (IRM) plans. So far, no cases of insect resistance to Bt maize have been reported anywhere.

An **EU Working Group on Insect Resistance Management** was formed in late 2001 to pro-actively prepare for large-scale cultivation of Bt maize in the EU. The Working Group represents an industry collaboration intending to harmonize the IRM plan for cultivation of Bt maize in the EU and other relevant European markets. This group was set up in light of the positive experience acquired in the USA with a comparable industry group, the Agricultural Biotechnology Stewardship Technical Committee (ABSTC) IRM Subcommittee.

The objectives of the EU Working Group are to:

(a) Assist in compliance with existing regulatory requirements (EU Directive 2001/18/EC).

(b) Take the lead in determining the requirements for monitoring of Bt maize, recognizing that the EU Commission has already considered some aspects of IRM such as developing a draft protocol for the monitoring of European corn borer resistance to Bt maize (EC Document XI/157/98).

(c) Protect Bt technology through good stewardship.

The Working Group currently involves the following industry partners: Monsanto Europe S.A., Pioneer Hi-Bred International, Inc. and Syngenta Seeds S.A.S.

2 SCOPE OF THE PLAN

The purpose of this harmonised IRM plan is to pro-actively avoid where possible, and in all cases delay the potential development of resistance to the Cry1Ab and Cry1F proteins, as expressed in Bt maize. The harmonised IRM plan also includes timely detection of changes in pest susceptibility and remedial actions in case of any confirmed development of resistance. The transformation events currently included in the proposal are presented in Table 1.

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

Transformation event	OECD unique identifier	Protein	Notifier
Bt 176	SYN-EV176-9	Cry1Ab	Syngenta
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

The success of the harmonised IRM plan is highly dependent on grower acceptance and implementation of the proposed management practices designed to preserve pest susceptibility to Bt proteins. In developing the IRM plan, the Working Group considered the latest scientific findings in order to both protect Bt technology and establish a practical, logistically achievable, approach that growers can follow.

The harmonised IRM plan is therefore based on published research (cited throughout the document), current EU legislation, the European Commission's Scientific Committee on Plants (SCP) opinion on IRM (SCP, 1999) and practical experience gained during the implementation of IRM plans in other parts of the world.

3.1 The European Union

3.1.1 Current legislation addressing monitoring

Directive 2001/18/EC, Annex VII, requires that notifiers develop and submit a monitoring plan together with the notification for placing on the market of a genetically modified (GM) crop. The objectives of the monitoring plan are 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 (e.r.a.) are correct and 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 e.r.a European Commission Scientific Committee on Plants (SCP).

3.1.2 European Commission Scientific Committee on Plants (SCP)

The European Commission's Scientific Committee on Plants (SCP, formerly known as Scientific Committee on Pesticides) has given a number of opinions on the cultivation of Bt maize in the EU. In their opinion of 09 December 1996 on the use of GM maize lines notified by Ciba-Geigy, the SCP stated that "Possible development of insect resistance to the Bt-toxin cannot be considered an adverse environmental effect, as existing agricultural means of controlling such resistant species of insects will still be available" (SCP, 1996).

In addition, the SCP has been consulted on the insect resistance monitoring program. After the first EU approval for Bt maize cultivation in 1997 (Bt 176), the European Commission's Directorate General for Environment (DG ENV) established an *ad-hoc* expert group consisting of representatives from EU Member States to develop a protocol to monitor the development of resistance to Bt proteins in target pests of maize. The 'Draft protocol for the monitoring of European corn borer resistance to Bt maize (EC Document XI/157/98)' was then forwarded to the SCP for a scientific opinion. The SCP opinion was expressed on 4 March 1999 with the following conclusions (SCP, 1999):

- The draft protocol to monitor for resistance in European corn borer to Bt maize should be linked firmly to field management plans on the ground. The proposed protocol, based on experience and practical data, should be broadened by the inclusion of more sensitive laboratory tests to detect low frequency resistance alleles and should include a suitable discriminating dose. Monitoring should be targeted to more extensive areas of planted Bt maize where selection pressures may be highest for resistance development and procedures adjusted according to the GM variety grown (in relation to seasonal decline of toxin concentration).
- Effective high-dose structured refuge resistance management plans should be implemented for Bt maize. Initially very large refuges may exist in practice, as the total area of Bt maize will be small in Europe. However, plans should also take account of spatial structuring on a local scale.
- Growers should be required to survey their growing Bt maize to detect unusual patterns of pest damage and ineffective control. Companies should thoroughly investigate these incidents by insect sampling and laboratory testing. Investigations should include the collection of plant material and the laboratory determination of expression levels in tissue, and the assessment of pest densities in the Bt crop and surrounding vegetation at the time of the problem.

3.1.3 Spain

GM maize expressing Cry1Ab protein (Bt 176) has been commercially grown in Spain since 1998 on approximately 20 – 25,000 ha annually. A detailed IRM plan was submitted to the hybrid registration authorities asking growers to implement the proposed IRM measures. Commercialisation of Bt 176 was accompanied by a monitoring research project to ensure early detection of European corn borer resistance through regular monitoring on Bt maize fields. Baseline susceptibility to the Cry1Ab toxin was also determined for Spanish populations of the ECB and MCB collected on non-Bt maize (Gonzalez-Nuñez *et al.*, 2000; De la Pozza *et al.*, 2001).

3.2 Practical experience worldwide

3.2.1 USA

With the registration of the first Bt maize in the USA in 1995 (Bt 176), it was foreseen that target pests increasingly exposed to Bt maize might develop resistance to Cry proteins. Measures such as the implementation of refuge zones constituted by non-Bt maize were envisioned to preserve the utility of the Bt technology. While prior to commercialisation an optimal refuge size and structure could not be determined by the registrants in the USA, it was thought that the market penetration of these crops would be sufficiently slow that considerable non-Bt maize remained to act as natural refuges as further research was conducted. Various voluntary refuge requirements were recommended. Efforts by both academia and industry were undertaken to develop a harmonised, science-based and practical approach to IRM. In 1997, a report was published on ‘Bt corn and European corn borer’ (Ostlie *et al.*, 1997)) and a resistance management plan for European corn borer were proposed (record where Bt maize is planted, implement 20-30 % refuge of non-Bt maize, monitor for product failure). In 1998, the United States Environmental Protection Agency (US EPA) began to institute refuge requirements for Bt maize.

In 1999, the industry ABSTC IRM Subcommittee, working with the National Corn Growers Association (NCGA) and in consultation with academia and the US EPA, proposed a harmonised IRM plan for Bt maize cultivation. With some modifications to this plan, US EPA put in place a consistent set of required refuge strategies for all Bt maize products. Beginning with the growing season in 2000, US EPA required a 20% non-Bt maize refuge to be planted within a half a mile of the Bt maize field (US EPA, 2001).

3.2.2 Argentina

Approval of the first Bt maize line in Argentina occurred in 1997 (Bt 176), followed by MON 810 in 1998. These products were introduced with a variety of voluntary IRM practices. In 1999, building upon the experiences in the USA, consultations began in Argentina among the Bt maize registrants on the possibility of developing a joint industry IRM plan. These consultations were held under the aegis of the Argentine Seed Association (ASA) and included third party entomologists from academia and the Instituto Nacional de Tecnología Agropecuaria (INTA).

This group proposed a harmonised plan with a 10% refuge requirement. The basis for this plan included knowledge of pest biology and 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 Biotecnología Agropecuaria (CONABIA) and implemented.

3.3 Current industry efforts in harmonisation of monitoring requirements and IRM

The Technical Advisory Group (TAG) of EuropaBio’s Plant Biotechnology Unit has developed a series of documents including monitoring of GM crops, to assist with harmonization regarding the technical information submitted to the EU regulatory authorities via applications for the commercial approval of GM crop products. Document 3.1 addresses the general approach to monitoring, and Document 3.2. discusses more specifically monitoring of insect-protected Bt crops (EuropaBio, 2001). In the case of existing Bt maize products, no adverse effects requiring case-specific monitoring have been identified in the environmental risk assessment (this conclusion is in agreement with the above mentioned SCP opinion (SCP, 1996)). However, to ensure good product stewardship, an Insect Resistance Management Plan for Bt maize is considered as critical by the industry. Therefore, since 2001, the work initiated by the TAG group has been specifically followed up by establishing the EU Working Group on Insect Resistance Management involving relevant industry partners.

4 CHARACTERISTICS OF THE IRM PLAN

For an IRM plan to be successful it must not only delay any development of pest resistance to Bt maize but also it must be effective, balanced and practical for the users such as 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 6 years, there has been no report of development of ECB resistance to Bt maize in the USA (Siegfried and Spencer, 2002).

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 will 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 companies subscribing to the present Working Group 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 four 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.
- 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 three 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 Bt crops is rare and genetically recessive. This has led to the development of IRM plans using an effective dose plus refuge strategy based on the following assumptions:

- Bt plants express high levels of Bt toxin
- 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 homozygotes that do not survive the Bt crop.

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 avoid where possible, and in all cases delay 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 the USA and Argentina is 20% and 10%, respectively. Such refuge sizes are considered to contain generous safeguard margins under the respective growing conditions, as described in Sections 3.2.1 and 3.2.2, respectively. A comparative analysis between agricultural landscapes in the USA and the EU highlights the fragmented and diverse cropping conditions in the EU. This explains why the current refuge requirements in the USA of 20% is considered highly generous for the EU (Appendix 1), thereby providing justification for a potentially lower level of refuge for the EU.

For the purpose of the present harmonised IRM plan for the EU, a grower is defined as the individual responsible for seed purchasing and planting decisions on one farm. 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. As a consequence, the requirement for refuge can only be applicable to farm sizes of more than 5 ha. The logistical barriers to implementing an effective refuge on small fields or farms and the reasons why the 5 ha threshold will not pose a resistance risk are outlined in Appendix 2.

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 (Appendix 3). 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 and Hunt *et al.* 2001, and this distance is 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 (see example in Appendix 3). Additional educational materials are addressed in Section 6 (see also NCGA point of purchase pamphlet in the reference list).

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 and control pest populations in non-Bt refuge maize only 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 monitoring

5.2.1 Objectives and underlying principles

The objectives of the resistance monitoring programme are to:

- Measure the baseline susceptibility of European corn borer (*Ostrinia nubilalis*, Hubner) and Mediterranean corn stalk borer (*Sesamia nonagrioides*, Lefebvre) to Cry1Ab (var. *kurstaki)* and Cry1F (var. *aizawai*) endotoxins of *B. thuringiensis*.
- Detect changes relative to baseline susceptibility that could result in inadequate protection against *O. nubilalis* and *S. nonagrioides* in the field.

The establishment of baseline susceptibility measurements for Bt maize will be an extensive and discrete effort. Subsequent monitoring for insect resistance will be an ongoing function that should be flexible and account for the factors that may result in the development of pest resistance to Bt maize.

In order to be effective, resistance monitoring should focus on areas of high selection for resistance, including those with the highest intensity of Bt maize. A regional or country-by-country component may be necessary; the former reflects biological reality and efficiency, and the latter the special needs of specific countries cultivating Bt maize.

It should be recognized that technical, biological and practical limitations exist. However, once baseline susceptibility is established, a monitoring programme designed as an integral part of the IRM plan, should provide the flexibility to increase monitoring in a certain area if the need arises. The extent and intensity of a resistance monitoring programme should therefore be balanced against other components of the IRM plan, such as specific efforts to survey and promote the implementation of IRM measures (e.g. refuge), or the monitoring of product adoption. For example, insect resistance monitoring could become more important if adoption of Bt maize is relatively high or if specific technical or practical challenges predict difficulties in reaching favourable levels of refuge implementation. The laboratory-based resistance monitoring efforts will be supported by a programme to follow product performance and unexpected damage.

5.2.2 Monitoring protocol

5.2.2a Baseline susceptibility

A number of studies have already been performed to measure baseline susceptibility (Marćon *et al*., 1999 and 2000; Gonzalez-Nuñez *et al.,* 2000 and Wu *et al.,* 2002). These have been conducted using different sources of protein and slightly varying methods. To reduce the level of variability and allow true data comparisons, it is important to perform the baseline susceptibility measurements for the EU in a coordinated manner using common sampling methods and sources of protein. A thorough report should accurately describe and contrast the baseline susceptibility of each individual population sampled. Any subsequent effort should follow the established methods and normalise the results obtained.

There are two general objectives of a baseline susceptibility study:

- To measure the susceptibility of target pest populations that may be exposed to Cry1Ab and Cry1F proteins in Bt maize.
- To measure the variability in susceptibility to Cry1Ab and Cry1F proteins that exists between ECB and MCB populations.

The former objective is used to determine the sensitivity of individual pest populations across geographic regions. The latter can be used to gauge the importance of potential shifts in susceptibility that may be detected in subsequent resistance monitoring efforts. A successful study of baseline susceptibility should carefully weigh the precision of estimates for individual populations against the scope or number of populations included in the baseline study.

The design of the baseline susceptibility study is a function of the factors that may increase the rate at which target pest species adapt to Bt maize. These factors are defined in insect biology and ecology, and will be linked to the distribution and intensity of Bt maize production throughout the EU. A summary of the important factors to be considered prior to the baseline susceptibility study is given in Appendix 4. Consideration of these factors suggests possibly four focussed regions based on where Bt maize cultivation could be greatest and where the intensity of target pest infestation could be relatively high.

The baseline study should determine the susceptibility of populations of target pests from three locations within each focus regions (e.g. a total of 12 populations for each target pest from the four focus regions). Additions or substitutions to these locations can be a function of target pest occurrence and feasibility of collection.

Participating companies will coordinate the insect collections. Collection efforts should focus on the egg stage for efficiency; however, any stage of insect may be collected to reach the required number of populations for each target pest.

Assays used to determine baseline susceptibility should be performed on F1 progeny whenever possible, and 200 to 300 insects should be collected from each sample location (population). Sample locations should be omitted if insect collections produce less than 100 individuals.

As recommended by the SCP (SCP, 1999), diet bioassays using F1 insects should be used to develop a 7-day LC_{50} and EC_{50}, and subsequently an LC_{99} and EC_{99} or a discriminating dose for use in future resistance monitoring. The methodology used in these assays should follow the methods described in the published work of Marćon *et al*. (1999, 2000) and Gonzalez-Nuñez *et al.* (2000). Specifically, the study should use seven to nine concentrations of each Cry protein (supplied by the Working Group) in a diet-overlay format. Estimates for each concentration should be based on no less than 60 total individuals using an appropriate experimental design with replication. When different events are expressing the same insecticidal activity, the baseline susceptibility measurements can be performed and applied to the different events using the same protein.

5.2.2b Resistance monitoring

Monitoring should be focused in areas where adaptation of target pests to Bt maize is more likely due to relatively high Bt maize cultivation in line with high target pest infestation.

The monitoring outlined below is designed to detect resistance when the frequency of the resistant allele reaches about 1-5%. For the proposed collection sizes, the upper 95% confidence limit for a dominant or partially dominant allele is 0.8%, while the corresponding 95% confidence limit for a recessive allele is 9%. However, these methods may not detect very small shifts in resistance allele frequency (ABSTC, 2002). Nonetheless, this level of sensitivity should allow for early detection of potential pest resistance before field failures occur and therefore would enable additional management measures to be effectively implemented in a timely manner.

Data generated during the baseline susceptibility study should be used to develop a technically accurate and more cost effective assay capable of detecting statistically and biologically significant changes in susceptibility within a population. A discriminating dose assay fits this goal, and the practicality of developing this assay should be evaluated after the baseline studies are complete (Hawthorn *et al*., 2001). Marćon *et al.* 2000 provides useful background on the discriminating dose assays.

As discussed earlier, the extent and intensity of resistance monitoring will be a function of the extent of Bt maize adoption and the intensity of target pest infestation. Geographically referenced measures of adoption will be performed as necessary and these measures will be inclusive of all Bt maize containing Cry1Ab or Cry1F and independent of company or brand name. Measures of adoption will be made at a resolution much smaller than the likely extent of the focus regions. This should increase the efficiency of the resistance monitoring programme. The analysis of adoption will be carried out by a third party at the expense of the Working Group. Results will be communicated to the Working Group and used to determine the necessary sampling efforts.

Insect collections will be coordinated by participating companies. Assays used to determine baseline susceptibility should be performed on F1 progeny whenever possible, and sample locations should be omitted if insect collections produce less than 100 individuals.

Tests for significant deviations from baseline LC_{50} and EC_{50} should be used for resistance monitoring until a discriminating dose assay is developed. The laboratory procedures for these LC_{50} and EC_{50} assays should be the same as those used in the measurement of baseline susceptibility.

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

5.3.1 Procedures for unexpected damage

The following procedures are proposed in case of unexpected damage:

a. The seed company will require distributors to instruct purchasers of Bt maize seed to report unexpected levels of damage caused by target pests.

b. The seed company will provide distributors specified information, including details of the report, grower contact details.

c. If the company is a licensee for the Bt trait, it will transmit this information to the notifier.

d. Companies will investigate the cause of these reports using available methods to confirm that the damaged plants express Cry protein, the damage resulted from a target pest and the damage is unexpected.

e. Insects will be collected for the purpose of further evaluation and confirmation subject to subsequent investigations to confirm that damage is unexpected.

5.3.2 Steps to confirm resistance

a. If damage is unexpected, the collected insects will be tested in a laboratory following specific guidelines used to confirm resistance.

b. Both of the following conditions must be met to confirm resistance: the collected insects or their progeny must have an LC_{50} that exceeds the upper 95% confidence interval of the historical (susceptible) mean LC_{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.

5.3.3 Remedial actions if insect resistance is confirmed

a. A remedial action plan will be developed, involving the relevant notifier and others concerned with the cultivation of affected Bt maize in collaboration with the pertinent Member State authority.

b. Components of an appropriate remedial action plan may include:

    - Informing customers and extension agent in the affected areas of confirmed resistance.
    - Increasing monitoring in affected areas.
    - Implementing alternative means to reduce or control target pest populations in affected areas.
    - Modifying and amending the IRM strategy accordingly.
    - If the above measures are not efficient, then cessation of sales in the affected and bordering areas may be necessary until an effective local management plan approved by the pertinent Member State has been put in place.

If interrupted, sale of Bt maize in the affected area will restart when an effective management plan has been implemented.

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 association as well as agricultural extension services web sites.
- Newsletters.
- Country specific hotlines.
- Relevant competent authorities.

A common outline of the IRM guidance is provided in Appendix 3 and will be adapted to the conditions of the local market.

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8 ABBREVIATION/DEFINITION OF TECHNICAL TERMS

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
LC_{50}	The median lethal concentration (i.e. the concentration/dose of substance that is estimated to be lethal to 50% of the test organisms)
LC_{99}	Lethal concentration (i.e. the concentration/dose of substance that is estimated to be lethal to 99 % of the test organisms)
MCB	Mediterranean corn stalk borer

9 APPENDICES

APPENDIX 1: *Comparative analysis of EU and USA agricultural landscapes*

Summary

A comparison of the landscape in the USA and the EU indicates that the adoption of a refuge strategy based on the USA model of 20% non-Bt maize refuge in Europe would result in an approach that incorporated highly generous safeguard margins for the harmonised IRM plan.

Paradigm

To base an IRM plan on the worst-case conditions with the highest probability to favour pest resistance to Bt crops will incorporate generous safeguard margins. These conditions correspond to where maize cultivation and Bt maize adoption are greatest and insect pressure is highest. In the USA, this occurs in Nebraska, Iowa, Minnesota, Illinois and Indiana, commonly referred to as the US Corn Belt. These five states routinely account for approximately 65% of the USA maize crop area and represent the conditions with the highest potential for the development of insect resistance. Conditions with highest probability of Bt maize adoption, and thus of potential resistance development, in the EU are limited to France, Spain, Germany and Italy, where approximately 85% of the EU maize is cultivated.

Comparisons of EU and USA agricultural landscapes

A comparison of these intensive maize-growing areas in the EU and the USA indicates that there are important differences between them that make the risk of resistance development significantly less for the EU than for the US Corn Belt. Examination of three key variables - land committed to agriculture, farm size and crop diversity – clearly demonstrates that the EU agricultural landscape is much more fragmented than that of the USA, thereby favouring greater durability of Bt maize in the EU.

- The US Corn Belt has an overwhelming 57 – 94% of its land committed to agriculture, whereas the four key maize-growing EU countries have between 49 and 60% of their land committed to agriculture (Table 1).
- France, Germany, Italy and Spain have an average of 10 to 20 times more farms per unit of farmland compared to the US Corn Belt (Table 1). A greater number of farms will result in increased crop diversity in an area.
- Maize is the major crop of the US Corn Belt and constitutes 18 to 40% of the total agricultural land and only slightly less of the total land area. However, maize constitutes only 2 to 11% of the total agricultural land of EU Member States. Moreover, non-maize cereal crops cover approximately 42%, 45% and 31% of the arable land in Germany Spain and France, respectively, and other important crops such as sunflower, potato and sugar beet also serve as alternate hosts for *O. nubilalis* (Hodgeson, 1928) (Table 2).

Conclusion

These data highlight the major differences between the EU and the USA maize-growing regions. These contrasting differences in farming practices, resulting in a much lower potential of risk resistance development, would suggest that an appropriate proportion of non-Bt maize refuge for the EU could be less than the 20% currently used in the US Corn Belt.

Table 1: Comparison of farm numbers and commitment of agricultural land for leading maize producing areas of the EU and the USA.

	Number of farms (x 1,000)	Total land area (1,000 ha)	% of land in agriculture
Europe:			
France	735	54909	55
Italy	2482	30132	56
Spain	1278	50488	60
Germany	567	35697	49
USA:			
Nebraska	55	19911	94
Iowa	96	14472	92
Minnesota	80	20621	57
Illinois	79	14399	78
Indiana	65	9290	68

Sources : Eurostat-Newcronos (1996) and USDA, NASS (2000)

Table 2: Summary of crop diversity for major maize-producing areas of the EU and the USA.

	Agricultural Crop Land (1,000 ha)																	
	Total Ag. Land Area	Corn (all)	Beans, Peas, Soy	Oats	Wheat (all)	Hay (all)	Barley	Rye	Oilseed Rape, Canola	Sunflower	Sugar Beet	Potato	Fresh Veg.	Grape	Citrus	Olives	Fruit Trees
France	30,060	3,307	2,786	170	5,115	.	1,534	41	1,369	799	461	171	321	873	3	13	172
Spain	30,126	545	83	409	2,423	.	3,107	122	48	850	.	136	388	1,166	288	2,350	979
Germany	17,344	1,698	706	309	2,601	.	2,210	748	1,198	33	515	309	90	101	.	.	55
Italy	16,743	1,314	349	142	2,388	.	350	5	51	209	.	86	.	908	182	1,154	.
Nebraska	18,785	3,439	1,902	53	708	1,254	4	18	.	38	32	.	.	.	.	.	.
Iowa	13,360	4,856	4,322	97	7	688	.	.	.	.	.	.	.	.	.	.	.
Minnesota	11,660	2,873	2,832	162	818	951	109	12	105	32	196	.	.	.	.	.	.
Illinois	11,215	4,471	4,228	22	372	343	.	16	.	.	.	2	.	.	.	.	.
Indiana	6,275	2,306	2,306	16	223	271	.	8	.	.	.	.	.	.	.	.	.

* Percent of total agricultural land area accounted for using these major and minor crops.
Sources: Eurostat 1995, 1999, USDA NASS 1999, 2000

APPENDIX 2: *Using an area threshold to achieve an effective and practical refuge*

Summary

The proposed refuge strategy for cultivation of Bt maize could consist of implementing a generous 20% refuge on farms where a Bt maize area greater than 5 hectares (ha) is cultivated. This strategy integrates the safeguards of a 20% refuge strategy in the European agricultural landscape (see Appendix 1) and the practical convenience to smaller scale maize farmers. More importantly, it also allows both large and small farmers to benefit from the cultivation of Bt maize without adversely affecting the risk of developing pest resistance.

Paradigm

An acceptable refuge strategy should balance effectiveness and practicality (Caprio *et al.*, 1999). Implementing an impractical refuge has at least two consequences. The first is grower reluctance or inability to plant a refuge that has unsatisfactory logistic or economic consequences to their production system. A second consequence is segmenting growers so that only some farm sizes can accommodate a refuge and the rest are unable to realize the economic and environmental benefits of Bt maize.

Three main considerations have been taken into account:

a. The negligible risk of developing resistance posed by the actions of small farm holders because of the very small total maize area that they represent.

b. The maize planting area below which implementing a generous 20% refuge becomes an economic and logistic hurdle for growers.

c. To ensure that efforts in terms of education are used efficiently.

5 ha area threshold

The 5 ha area threshold was determined based on:

a. *The agricultural landscape*. Based on the four major maize-growing countries in the EU (France, Germany, Italy, Spain), it can be inferred that:

   - Only a small proportion of the total maize area in the EU is cultivated on small farms (less than 5 ha): in France, maize grown on small farms amounts to 8%, in Germany 1%, in Italy 19% and in Spain 8% (Tables 1 and 2).
   - However, the "less than 5 ha" farms represent a significant proportion of the maize farmers in the EU: in France 35%, in Germany 5%, in Italy 75% and in Spain 47% (Tables 1 and 2).

   This agricultural structure implies that the farming area, even specifically considering maize, is very fragmented (for the general farming area fragmentation see Appendix 1).

b. *The agricultural practices*. It would not be economically sustainable for most of the growers cultivating less than 5 ha of Bt maize to adopt a 20% refuge strategy because:

   - Most maize seed is distributed in the EU using bags containing 50,000 to 80,000 kernels per bag, and seeding rates usually range from 70,000 to 80,000 kernels per hectare
   - Maize producers usually spread the risk of an unpredictable growing season by planting more than one seed product (hybrid name).

Planting a 20% refuge would in most cases force growers to buy excess seeds and would be detrimental to their business.

c. *The negligible risk of resistance*. The proportion of less than 5 ha Bt maize area represent a minority of maize land area with an equivalently small amount of potential selective pressure because:

- The fragmentation increases the probability that small maize fields will be bordered by other crops, weedy/grass barriers or fallow land
- The probability that target pests will routinely immigrate from multiple over-wintering sites (previous maize) to maize planted during the following season will be increased (Cordero *et al.,* 1998; Hellmich *et al.,* 1998; Losey *et al.,* 2001).

Also, it can be expected that farms cultivating less than 5 ha of Bt maize will benefit from the presence of non Bt maize on neighbouring farms which either did not adopt the Bt technology or where refuges are implemented at the generous 20% level (the majority of the maize planting area). Therefore, it is reasonable to consider that both the proportion and the fragmentation of the less than 5 ha Bt maize area within the overall maize cultivated area in the EU makes it highly unlikely for them to contribute to the development of pest resistance.

Since the risk of developing resistance is negligible, educational and monitoring efforts could be better targeted to areas of Bt maize where there might be a potential for development of pest resistance. Those efforts will much more efficiently be used to educate and survey the larger growers that contribute to the great majority of maize production in the EU.

Conclusion

Effective and practical IRM can be achieved by adopting a generous 20% refuge on farms planting Bt maize areas greater than 5 ha without compromising the potential for development of pest resistance. This represents the largest and most relevant proportion of farmers for education on insect resistance management, the largest fraction of the cultivated maize land area, and the farms that are most likely to impact the potential development of pest resistance to Bt maize. In addition, the 5 ha area threshold for farms provides a practical means of accommodating existing agricultural and business practices without compromising the effectiveness of the refuge strategy.

Table 1: Distribution of maize cultivation over size classes of farm holdings for the four largest maize-producing EU Member States.

Distribution of Maize Cultivation Over Farm Sizes

France, cultivation for grain

Farm size	< 5 ha	5 - 10 ha	10 - 20 ha	20 - 30 ha	30 - 50 ha	> 50 ha	Total ha
Number of farms	60,793	29,224	27,099	11,363	8,957	5,265	142,701
Number of farms %	43%	20%	19%	8%	6%	4%	100%
Area ha	132,700	205,771	377,469	274,463	338,852	424,611	1,753,866
Area %	8%	12%	22%	16%	19%	24%	100%

France, cultivation for silage

Number of farms	42,735	35,927	42,241	11,612	3,902	466	136,883
Number of farms %	31%	26%	31%	8%	3%	0%	100%
Area ha	105,078	257,719	583,269	272,539	138,109	28,214	1,384,929
Area %	8%	19%	42%	20%	10%	2%	100%

Germany, cultivation for grain

Farm size	< 2 ha	2 - 5 ha	5 - 10 ha	10 - 20 ha	20 - 30 ha	30 - 50 ha	50 - 100 ha	> 100 ha	Total ha
Number of farms	313	2,700	4,739	7,846	5,598	8,851	8,670	2,974	41,691
Number of farms %	1%	7%	11%	19%	13%	21%	21%	7%	100%
Area ha	144	3,956	11,571	30,899	31,859	75,447	121,141	95,719	370,735
Area %	0%	1%	3%	8%	9%	20%	33%	26%	100%

Germany, cultivation for silage

Number of farms	126	3,865	10,580	26,606	23,442	33,088	29,261	10,894	137,862
Number of farms %	0%	3%	8%	19%	17%	24%	21%	8%	100%
Area ha	74	4,007	15,003	71,784	105,365	239,159	334,796	432,655	1,202,844
Area %	0%	0%	1%	6%	9%	20%	28%	36%	100%

Italy, cultivation for grain

Farm size	< 2 ha	2 - 5 ha	5 - 10 ha	10 - 20 ha	20 - 30 ha	30 - 50 ha	50 - 100 ha	> 100 ha	Total ha
Number of farms	122,557	80,549	42,460	27,673	13,055	10,150	5,772	2,837	305,053
Number of farms %	40%	26%	14%	9%	4%	3%	2%	1%	100%
Area ha	84,489	119,920	127,947	151,386	103,059	102,600	127,220	108,627	900,328
Area %	9%	13%	14%	17%	11%	11%	11%	12%	100%

Italy, cultivation for silage

Number of farms	12,350	3,100	4,350	2,989	1,219	1,028	1,064	503	26,602
Number of farms %	46%	12%	16%	11%	5%	4%	4%	2%	100%
Area ha	9,500	15,300	40,700	59,800	44,700	41,100	56,200	60,300	327,600
Area %	3%	5%	12%	18%	14%	13%	17%	18%	100%

Spain, cutlivation for grain and silage

	< 2 ha	2 - 5 ha	5 - 10 ha	10 - 20 ha	20 - 30 ha	30 - 50 ha	50 - 100 ha	> 100 ha	Total ha
Number of farms	30,915	35,912	24,242	23,158	8,657	7,393	6,910	5,586	142,772
Number of farms %	22%	25%	17%	16%	6%	5%	5%	4%	100%
Area ha	10,349	28,446	36,914	75,787	58,604	59,603	82,516	155,670	507,889
Area %	2%	6%	7%	15%	12%	12%	16%	31%	100%

Sources: France- Monsanto on ISTAT Source, Germany- Statistisches Bundesamt 1999, Italy- , Spain- .

Italy – Monsanto on ISTAT Source

Table 2: Contribution of small farms to the extent of maize grown in the EU (extracted from Table 1)

	% of small farms* planting maize (average)	% of maize planted on small farms* (average)
France	35	8
Germany	5	1
Italy	75	19
Spain	47	8

** Farms of less than 5 ha*

APPENDIX 3: *Proposal for grower information material*

INSECT RESISTANCE MANAGEMENT INFORMATION SHEET

Bt Maize

Bt maize has proven to be an important technology to help maize growers control damaging insect pests and produce higher yields and better quality grain.

Insect Resistance Management (IRM)

To preserve the many benefits of Bt maize technology, the implementation of an IRM plan is essential. An effective BT maize IRM plan includes the planting of a non-Bt refuge (a block of non Bt maize) planted close to your Bt maize crop.

All Bt maize designed to control European corn borer and Mediterranean corn borer require implementation of an IRM program according to the refuge size, distance guidelines and insecticide usage described in this information sheet.

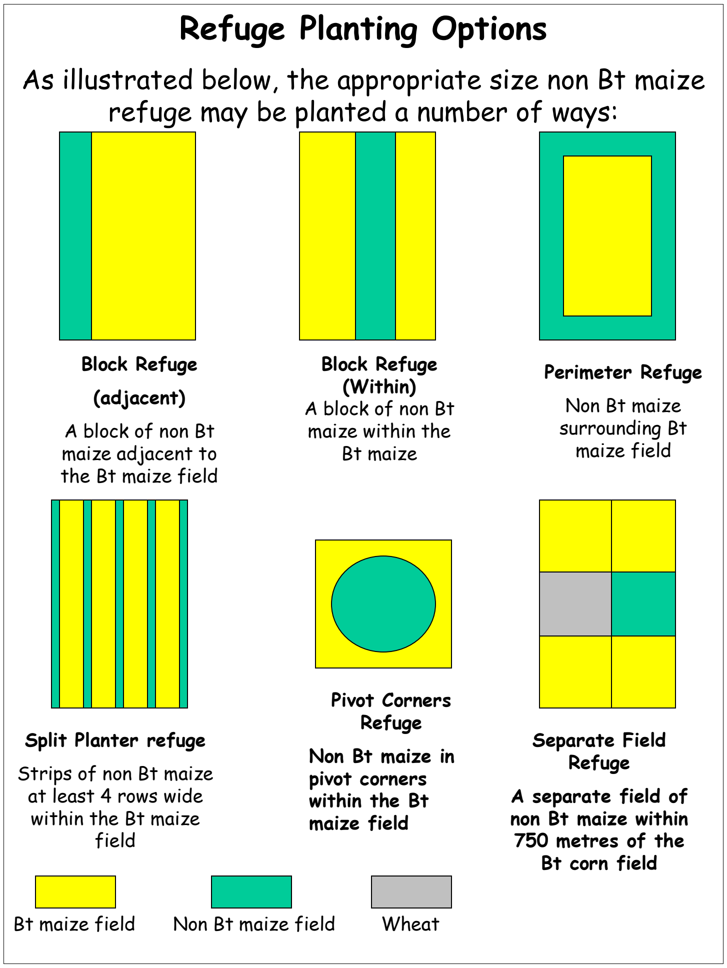
Refuge size requirements

If you plant greater than 5 hectares of Bt maize, total within or among fields you must plant a refuge.

For each sowing, plant at least 2 hectares of non Bt maize for every 8 hectares of Bt maize (minimum 20% non Bt refuge, maximum 80% Bt maize)

Refuge Distance Requirement

A non Bt maize refuge must be planted within 750 metres of each Bt maize field


Refuge Planting Options
As illustrated below, the appropriate size non Bt maize refuge may be planted a number of ways:
Block Refuge (adjacent)
A block of non Bt maize adjacent to the Bt maize field
Block Refuge (Within)
A block of non Bt maize within the Bt maize
Perimeter Refuge
Non Bt maize surrounding Bt maize field
Split Planter refuge
Strips of non Bt maize at least 4 rows wide within the Bt maize field
Pivot Corners Refuge
Non Bt maize in pivot corners within the Bt maize field
Separate Field Refuge
A separate field of non Bt maize within 750 metres of the Bt corn field
Bt maize field
Non Bt maize field
Wheat

Insecticide Use in Bt and Non Bt refuge

Your Bt maize and non Bt maize refuge may be treated with conventional insecticides ONLY if the target pest populations reach economic thresholds.

Microbial Bt insecticides must not be used within the refuge.

Refuge Management

In order to maximise the effectiveness of the refuge, you should manage your non Bt maize and the Bt maize in a similar manner. This can be accomplished by planting your non Bt maize as close to and at the same time as you Bt maize. In addition, select non Bt hybrids and Bt hybrids that have similar growth and development characteristics.

APPENDIX 4: *Determining sample number and distribution for baseline susceptibility studies and subsequent monitoring*

The sample number and distribution of samples included in the baseline susceptibility study are dependent on the factors that could increase the probability of insect adaptation to Bt maize. The predictive factors used in this proposal are geographic and biological. Geographic variables include: the total land area routinely planted to maize, the concentration of maize cultivation within and among regions, and geological barriers to panmixia. Biological variables include: the annual intensity of target pest infestations, the seasonal cycle of pests within a region, and our best information on the biology and ecology of target pests. Integrating these factors does not define the probability, but provides an indication of the relative likelihood of adaptation in different regions, and this is a useful guide for the administration of resistance monitoring resources.

Table 1 describes the distribution and concentration of maize production among the Member States. In general, the probability of insect adaptation to Bt maize should be proportional to the land area planted to Bt maize, the concentration of Bt maize in a particular region, and the abundance of the target pest.

Table 1: Distribution and concentration of maize production among the Member States

EU Member State	Total land area (in 1,000ha)	Arable land area (in 1,000ha)	Maize land area (in 1,000ha)	Maize concentration (% of arable land)	Sources for maize land area
France	54909	18480	3260	17.6	SCEES, 2001
Germany	35697	11801	1517	12.9	DMK, 2001
Italy	30132	8192	1334	16.3	Consorzio Italiano per il Telerilevamento in Agricoltura, 1999
Spain	50488	12884	559	4.3	Ministry of Agriculture, 1999
Austria	8386	1396	248	17.8	Agrarmarkt Austria, 1999
Netherlands	4153	977	246	25.2	National Statistical Institute (CBS), 2001
Belgium	3052	852	221	26.0	National Statistical Institute (NIS), 2001
Portugal	9191	2096	138	6.6	AMIS Seed, Kleffmann (National Statistics & Seed Industry Estimates), 2001
Greece	13196	1981	130	6.5	AMIS Seed, Kleffmann (National Statistics & Seed Industry Estimates), 2001
UK	24410	6625	101	1.5	Eurostat, 2001
Denmark	4309	2364	79	3.3	Eurostat, 2001
Ireland	7029	1049	18	1.7	Teagasc, 2001
Luxembourg	257	60	11	18.3	National Statistical Institute (STATEC), 2000
Finland	33815	2143	-	-	Eurostat, 2001
Sweden	44996	2745	-	-	Eurostat, 2001

Total land area and arable land area sources: EuroStat 2001

Spain, France, Germany and Italy contain about 85% of all maize land area in the EU and in these countries maize is grown on 4 to 18% of the total arable land. The remaining countries have significantly smaller total areas routinely planted to maize and the concentration of maize on arable land varies greatly among these countries.

The distribution of maize in dominant maize-producing countries is not evenly distributed, but is aggregated in regions within countries. This aggregation suggests that resistance monitoring efforts initially should focus on (but not necessarily be limited to) possibly four regions within the EU where Bt Maize is likely to be cultivated. These regions will be defined as necessary to maximize the efficiency of the monitoring programme.