ECOLOGY

Assessing and monitoring impacts of genetically modified plants on agro-ecosystems: the approach of AMIGA project

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Abstract

The environmental impacts of genetically modified crops is still a controversial issue in Europe. The overall risk assessment framework has recently been reinforced by the European Food Safety Authority

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(EFSA) and its implementation requires harmonized and efficient methodologies. The EU-funded research project AMIGA – Assessing and monitoring Impacts of Genetically modified plants on Agro-ecosystems – aims to address this issue, by providing a framework that establishes protection goals and baselines for European agro-ecosystems, improves knowledge on the potential long term environmental effects of genetically modified (GM) plants, tests the efficacy of the EFSA Guidance Document for the Environmental Risk Assessment, explores new strategies for post market monitoring, and provides a systematic analysis of economic aspects of Genetically Modified crops cultivation in the EU. Research focuses on ecological studies in different EU regions, the sustainability of GM crops is estimated by analysing the functional components of the agro-ecosystems and specific experimental protocols are being developed for this scope.

Introduction

Since the first wide-scale planting of genetically modified (GM) crops in 1995, the global surface devoted to these crops has expanded rapidly worldwide with about 170,000 ha grown in 2012 (James, 2012). Major GM crops to date include maize, cotton, soybean and canola. In the EU, 114,490 ha of GM crops were grown. So far, only three GM plants, maize MON810, maize T25 and potato EH92-527-1 (BASF Amflora), have been approved for cultivation, while several other GM plants have been approved for import or await their approval for cultivation (http://ec.europa.eu/food/dyna/gm_register/index_en.cfm).

Within the EU, the environmental risks associated with GM plants still remain a controversial issue. Environmental risk assessment (ERA) models for GM organisms can be informed by conventional eco-toxicological models (Romeis *et al.*, 2008), exotic species models (Orr *et al.*, 1993) or ecological models (Andow & Hilbeck, 2004). Recently, the GMO Panel of the European Food Safety Authority (EFSA) proposed a risk assessment approach for the European environments based on the selection of focal species representative of functional groups within a tiered approach (EFSA, 2010b). This approach openly utilizes some of

the elements from a range of existing approaches. The main goal is the analysis of functional biodiversity in agro-ecological habitats and the possible interference of its normal functioning caused by GMPs.

Risk assessment requires scientific data about the possible environmental impacts of cultivation of GMPs. There is a general consensus about the need for research that is directly related to specific European environments and agricultural settings (*e.g.* Jesse and Obrycki, 2003; EFSA, 2010b). In fact, due to the longer experience of cultivation of GMPs in other geographical areas (particularly in the Americas and China) the existing studies on ERA for GMPs are mainly based on indicator species and agricultural practices appropriate for those areas. For example, the butterfly species *Danaus plexippus* L is an important species for estimating environmental effects of Cry1Ab-expressing maize to non-target Lepidoptera in North America (Lövei & Arpaia, 2005). However, the sensitivity of European butterfly species, their life cycles, ecology and their possible exposure to Cry-expressing plant parts is in some cases very different in Europe and attempts to directly extrapolate results from the *Danaus* model to European environments are problematic or even irrelevant (Birch & Wheatley, 2005; EFSA, 2009; Lövei *et al.*, 2009). Indeed, recent data suggest that the sensitivity of European species to various Cry toxins is different when compared to this *surrogate* species (EFSA, 2011).

Due to the high diversity of receiving environments, as well as of management systems, the ERA should consider a range of representative scenarios but at the same time cannot cover all possible situations. Also, receiving environments and management systems are continuously changing. In this context, Post Market Environmental Monitoring (PMEM) is of major importance in validating the hypotheses posed during the ERA, to ensure that the deployment of the GM plant *falls within the domain of validity of the conclusions of the ERA*, to detect any unexpected adverse effects, and to mitigate risks through the development of novel management systems which are fine-tuned for specific crops, regions and agronomic practices.

The ERA Guidance Document (EFSA, 2010a) sets out a number of particular areas of potential environmental concerns for applications relating to GM crops: i) impacts on non target organisms (NTOs *e.g.* natural enemies and pollinators; Arpaia, 2010); ii) impacts of GM crops on soil biodiversity and biology; iii) long-term effects on the environment, biodiversity and biogeochemical cycles; iv) impact of farming practices, with the need to take into account the diversity of management systems that may be associated to GM crops.

Statistical procedures are required in order to support the ERA with robust data analyses. Finally, the European Commission has requested that PMEM guidelines should be updated to strengthen the overall decision-making process, by incorporating long term effects of GM crops and related agronomic changes relative to the current baseline of agricultural practices over the last 30 years as a mechanism for estimating rates of change.

All these aspects require the development of feasible interdisciplinary research and science-based methods, which meet the expectations of decision-makers and society. The recently approved EU-project AMIGA (Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems) is addressing these challenges and in particular the perceived lack of standardized approaches, methods and protocols. The research being undertaken in AMIGA project is therefore fundamental to move towards a new understanding of the potential benefits and risks associated with the field cultivation of GM crops in the EU.

AMIGA objectives

The AMIGA project aims to: i) *reduce uncertainty* in decision-making for the cultivation of GMPs in Europe by developing and verifying

robust Environmental Risk Assessment methods based on testable hypotheses that seek to aid the decision making process; ii) *increase confidence* in the practicability of EFSA Guidance Documents for Environmental Risk Assessment; and iii) *contribute to the development* of more effective PMEM designs and risk mitigation procedures.

The specific objectives are: i) *to provide baseline data on biodiversity in selected agro-ecosystems and adjacent habitats* through field surveys in non-GM cultivations in five different geographical European regions and build a database of species assemblages and ecological functional groups in the chosen crops (maize and potato); ii) *to aid the translation of regional protection goals into measurable assessment endpoints* by establishing a link between functional groups of arthropod species active in two selected agro-ecosystems (maize and potato), whilst taking into account the differences in the ecology of the different agro-ecosystems; iii) *to outline potential bioindicators* (species assemblages and/or ecosystem functions) *fine-tuned for specific European regions* and translate them into a decision matrix, which can provide guidance on the selection of relevant test species, experiments and monitoring targets/areas; iv) *to improve knowledge on the potential long-term environmental effects of GMPs* by assembling background information on changes in land use, cropping practices and the economic returns of arable farming for representative crops and areas in different regions (over the last 30 years) in order to help assess future effects of GM-based agro-ecosystems in the context of impacts due to other factors; v) *to provide examples for testing the efficacy of the EFSA Guidelines for the ERA of GM plants* and *produce standardized protocols* for ERA in laboratory as well as field conditions; vi) *to explore possible strategies for data-driven post market monitoring of GMPs* and develop a coherent framework, validated methods and tools, including exposure models, to help implement cost-efficient monitoring schemes; vii) *to estimate the compatibility and sustainability of GM crops within the principles of Integrated Pest Management* which represent the sole pest management options in the EU after 2014; viii) to evaluate the potential risks of GM crops for *non-target pollinators and crop pollination services;* ix) *to Provide a systematic analysis of economic aspects of cultivation of GMPs*; x) *to provide a training and communication plan* that addresses current public concerns about GMPs in Europe.

AMIGA research approach

In relation to the above objectives, the AMIGA project is undertaking a range of research studies in the following areas.

Selecting receiving environments to support the environmental risk assessment

Several biogeographical zones have been identified in Europe, based on climatic and ecological criteria. Some areas are also considered biodiversity hotspots (*e.g.,* the Balkans and the Mediterranean). As Europe is a continent with long history of human management, the environment-management interface is very variable in rural areas, due to different agricultural conditions and practices. There are many climatic, ecological, agricultural and political mechanisms for defining geographical regions or zones in Europe (EFSA, 2010b), although the defining of geographical zones based on a sound scientific rationale, taking into account realistic agricultural situations, will be the most valuable and consistent for GM risk assessment. Past GM risk assessments in Europe have not explicitly considered such differences, exposing the risk assessment practice and its outcomes to criticism. The EFSA ERA Guidance Document (EFSA, 2010b), considers four classifications of geographical zones in Europe, but gives no clear instruction or standard on how to adopt them. AMIGA therefore aims to vali-

date with real case studies, a logical road map for the selection of bio-geographical zones.

Further, through literature and ecological surveys conducted in 5 geographical zones in potato and maize growing areas, AMIGA research will identify region-specific characteristics of these agro-ecosystems and provide a decision-support tool for further supporting a correct selection of receiving environments. These will be reflected in databases of species assemblages to be used in the definition of geographical regions to be adopted for ERA of GM maize and potato in Europe and in a decision matrix to support guidance for any GM crop.

Assessing possible effects on natural enemies

Natural enemies such as parasitoids and predators (Figure 1) represent an important functional guild of NTOs which provide a key *ecological service* to farming activities (Arpaia, 2010). Several serious shortcomings in the current risk assessment tests for non-target organisms have been identified (Lövei & Arpaia, 2005, Charleston & Dicke, 2008). Firstly, the choice of measurement endpoints is often limited to the detection of acute short-term single toxic effects, and sub-lethal effects, longer-term or combined effects were ignored (Whitehorn *et al.,* 2012). While mortality provides a measurable life history factor, it is important to consider that sub-lethal effects alone can also drive a population to extinction (Hallam *et al.*, 1993). A meta-analysis of results from laboratory studies has demonstrated that mortality might not even be the most sensible indicator of adverse effects (Lövei *et al.,* 2009). Therefore, other measurement endpoints such as development, growth, fecundity, fertility, behavior, etc. need to be considered as predictors of possible environmental effects.

Testing for sublethal effects is important since it can give indications of possible long-term impacts. For instance, an appropriate measurement endpoint for NTO testing is relative fitness, which is the relative lifetime survival and reproduction of the exposed versus unexposed non-target species (Birch *et al.,* 2004).

AMIGA is performing NTO studies through bi-trophic and tri-trophic *in planta* tests, considering toxic effects (short-term mortality, longevi-ty) as well as reproductive parameters (*e.g.* number and size of offspring, percentage of eggs hatching, sex ratio of progeny, age of sexual maturity), growth and developmental rate and, when appropriate, behavioural characteristics (*e.g.* searching efficiency, predation rates, food choice). Tritrophic studies are the most suitable method to detect host-quality mediated effects, which are thought to be a relevant exposure route for natural enemies (*e.g.* Andow *et al.*, 2006; Naranjo, 2009).

The selection of *focal* species and ecological functions as potential bioindicators of environmental effects will be conducted according to crop-trait-environment and agricultural practices in the selected areas as indicated in the EFSA GD (2010b).

Describing the biological components of soil fertility through advanced methods

An important ecosystem service associated with sustainable land use is creating and maintaining soil fertility, which is largely influenced by soil inhabiting organisms. These ecosystem service providers come from many different biological taxa, ranging from microorganisms to earthworms; co-operatively they provide key functions, *i.e.*, fixation and mobilization of plant nutrients, degradation of plant residues, biogeochemical cycling of elements or control of pests (*e.g.*, Bardgett, 2005, van der Putten *et al.*, 2001).

The detection of the potential adverse effects of GM crops on soil organisms is hampered by several obstacles. Due to their high diversity, their characterization is laborious, and requires expert knowledge. Furthermore, most soil-inhabiting organisms are highly sensitive to crop species, agricultural management practices, landscape factors, and weather conditions. This causes a high level of variability that may mask effects triggered by GM-plants. A certain response of soil organisms to a GM crop can only be interpreted against this background of natural variability, as it occurs with other crops, tillage practices or environmental triggers. Furthermore, changes must take into consideration temporal and spatial scales in order to allow conclusion on adverse effects on soil fertility.

AMIGA aims to produce new scientific data on GM crop interference with soil fertility by establishing baselines for microbial diversity through advanced DNA-sequencing methods, *via* soil metagenomics.

These methods allow the accurate measurement of unanticipated effects of GM crops on non-target microorganisms and nematodes and biogeochemical cycling, as required in the context of an application for cultivation in Europe. The methods provides a robust empirical basis for risk assessors, and therefore potentially increase the environmental safety of GM crops.

Designing methodologies to assess effects of genetically modified crops on pollination ecosystem services

Pollinators provide a *key ecosystem service* that is crucial to the maintenance of both wild plant biodiversity and agricultural productivity. The worldwide economic value of insect pollination could be as high as 153 billion euros/year (Gallai *et al.,* 2009) and 65% of the 134 main crops used directly for human consumption in the world are dependent on insect pollinators, particularly bees (Klein *et al.,* 2007; Potts *et al.,* 2010). Consequently, *bee pollinators* (Figure 2) are a group of key test species for assessing the impacts of GM crops on non-target organisms (Duan *et al.,* 2008; Malone & Burgess, 2009).

The honey bee, *Apis mellifera* is the principal bee species mainly used and managed for crop pollination (Potts *et al.,* 2010), but other wild pollinators also provide significant crop pollination services (Garibaldi *et al.,* 2011). Domesticated, feral or wild *A. mellifera* populations are present in all countries growing GM crops. Despite the importance of bees as pollinators, their foraging distances and population dynamics in the context of agricultural intensification, GM crops and landscape-wide

Figure 1. *Adalia bipunctata* L. is an important predator species supporting the natural control of aphids in many European environments.

crop rotation patterns have rarely been studied, (Steffan-Dewenter & Kuhn, 2003; Holzschuh *et al.,* 2012). Further, standardized methodologies to test the single and combined effects of GM crops, pesticides and pathogens on honey bees and other non-managed social and solitary bee species are required (Hendriksma *et al.,* 2011).

AMIGA will assess the potential impacts of GM crops on pollinators and crop pollination services by implementing a tiered approach which combines experiments in confined environments, field studies and landscape-scale approaches (Figure 3). Overall, AMIGA research is being undertaken at different scales, including data-based synthesis work via model development. AMIGA is thus producing data and methods which will make it possible to evaluate GM effects on wild and managed pollinators and the important ecosystem service of pollination at an appropriate European scale.

Providing a methodological framework for assessing long-term effects

Long term effects of any change to a cropping system, such as the introduction of a GM crop and its associated management, originate either from a response that takes time to emerge to a detectable level from the background noise, but is in principle measureable by experiment, or from the inevitable increase in complexity that occurs after release due to increase in the number of interactions between the GM crop and its biophysical environment. This second form of effect is inherently difficult to predict from short-term field trials and will be tackled in AMIGA by reference to existing information on the long term effects of other changes to cropping systems in recent decades.

There are five steps to the process: i) The main trends will be defined in descriptors such as the areas cropped, yield, and inputs of fertilizer and pesticide over the past 30 years. Such trends and their inter-annual dynamics should reveal the resistance and resilience of cropping systems to major change. ii) Models will be applied to standardize and compare such changes between regions in terms of the common *currencies* of energy, carbon and nitrogen. iii) A set of around 100 indicators, or measurable, will be chosen that define the state of a cropping system and that can be used to link the performance of crops in GM experiments to the main trends referred above. iv) The indicators will be used to set safe ecological limits for the main processes and variables, such as flows in the carbon and nitrogen cycles, crop growth and yield and trophic interactions. v) The final and most complex step is to assess from experimental and historical evidence whether GM crops are likely to shift the current cropping systems in relation to these safe ecological limits. It should then be possible to say whether propo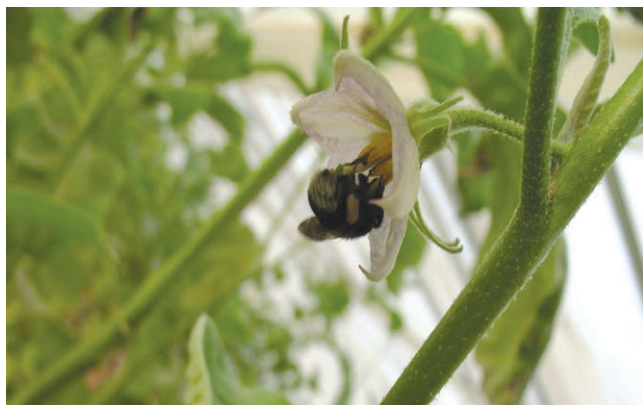sed innovations (GM or otherwise) are consistent with management strategies that aim to make agriculture more resilient, ecologically safe and ultimately sustainable. The outcome will be a detailed but practical framework and methodology for examining long term effects as well as a central database that could be periodically updated.

Developing a framework for an efficient integration of environmental risk assessment and post-market environmental monitoring

In reference to Directive 2001/18/EC, a Post-Marketing Environmental Monitoring (PMEM) is introduced in order i) to verify the hypotheses made during the ERA, ii) to ensure that the deployment of the GM plant *falls within the domain of validity of the conclusions of the ERA*, iii) to assess possible effects that may appear only where releases are of a large-scale, iv) to assess potential cumulative long-term effects, and v) more generally, to detect any unexpected adverse effects.

Over the last ten years, there have been numerous contributions on the practical implementation of PMEM (*e.g.* Sanvido *et al.,* 2005; EFSA, 2006, 2011; Marvier *et al.,* 2008; Beckie *et al.,* 2010), specialized workshops, as well as attempts to derive a harmonized approach among Member States. It is widely recognized that the current implementation does not fully meet the regulatory requirements and that further guidance is required.

AMIGA aims to provide further guidance by: i) defining baselines of the various receiving environments into which GM crops may be grown to serve as a point of reference against which any effects arising from the placing on the market of a GM crop can be compared; ii) designing generic exposure models taking account of landscape patterns and variability of management systems; spatially-explicit exposure models are being developed to assess the impact of GM-based cropping systems on weed diversity as well as to assess impacts of Bt crops on non-target organisms; iii) designing a dynamic information system that assists decision-makers analyze the outcomes of monitoring studies in the context of continuously evolving receiving environments, context and management systems.

This approach should help to *identify hotspot situations* and drive sampling monitoring schemes by targeting specific situations where potential adverse effects are most likely to occur (Holst *et al.,* 2013).

Exploring the potential of genetically modified crops to support integrated pest management

Despite increasing inputs of pesticides over the last 40 years (7-fold increase in pesticide use; Tilman *et al.,* 2001), current global crop losses show no significant reduction. GM crops offer potential advances in our ability to manage agricultural pests (including diseases and

Figure 2. Bumblebee (*Bombus terrestris* L.) foraging on sunflower.

Figure 3. Agricultural landscape in Central France.

weeds) safely and effectively, if management is tailored to regionally variable agro-ecosystems. The importance of integrated pest management (IPM) for GM crops has been repeatedly stressed (*e.g.*, Hokkanen & Wearing, 1994; Birch & Wheatley, 2005, Romeis *et al.,* 2008); because their effectiveness in controlling pests is under pressure from insect, disease and weed evolution, appropriate IPM practices can extend the usefulness of specific events.

Under the new Directive 2009/128/EC, all EU Member States (MSs) are required to draw up National Action Plans in order to implement IPM by 1 January 2014, and shall take all necessary measures to promote and support low pesticides-input pest management. However, no comprehensive IPM programs have been developed to accompany the introduction and use of GM-crops. AMIGA research is assessing the impact of the new regulation on GM crops as well as the potential of GM crops to support IPM systems. Thus the AMIGA GM field studies (maize, potato) include both the individual component strategies, as well as IPM systems.

Designing and analyzing field trials for environmental risk assessment of genetically modified organism

The most relevant elements of a desirable statistical approach to environmental risk assessment are indicated in the EFSA GD (Perry *et al.,* 2009; EFSA 2010a) and they include: i) simultaneous use of difference and equivalence testing, ii) experimental design based on *a priori* power analysis of difference tests, iii) testing based on additive models for transformed data, and iv) statistical models that should include genotype by environment interactions. However the approach is currently limited to data following a normal or log-normal distribution, and most guidelines are qualitative rather than quantitative. AMIGA is further developing the EFSA statistical guidelines to overcome some of these limitations and will complement the existing protocol with software for performing power analysis, for fitting statistical models and for reporting and displaying the results of the analysis.

Assessing the economic impacts of genetically modified crops

The direct economic consequences of growing transgenic crops still requires further exploration and updating. Much of the economic evidence related to the commercial growth of these crops is based on experiences outside of the EU, where the systems and rotations into which they are incorporated are different. The project has undertaken a thorough review of the literature as a basis for constructing a broad based economic model capable of exploring the economic impacts of including GM crops within existing agricultural systems within the EU. This builds on previous research which suggests, that with the events currently available, the economic impacts would be relatively small (Park *et al*., 2011). However as with other technical innovations in agriculture, the introduction of GM crops are likely to have wider implications for the agro-ecosystem which in turn can have a range of secondary impacts, which may be beneficial or detrimental. These will be investigated within the AMIGA modeling approach.

Setting a training and communication plan that addresses current public concerns about genetically modified plants in Europe

Sound scientific communication is an essential part of the work to be done on potentially controversial and sensitive issues such as GMOs in Europe. The AMIGA team is well placed to reach policy and research stakeholders in the field of GMO crops, as well as the public-at-large with targeted communication strategies. The AMIGA communication plan addresses in particular the public concerns regarding GMO cultivations and any other type of environmental releases, including specif-

ic measures to detail the main controversies, and explaining scientific context in a clear but simplified way for the general public.

Further, AMIGA will i) provide practical opportunities to junior researchers to familiarize themselves with the state-of-the-art through workshops and Summer Schools, ii) provide a forum for discussion of GMs in the higher education context, and iii) develop teaching materials that can be utilized within higher education institutions.

AMIGA organization

The project team includes a wide geographical representation of European cultivation areas. Participants to the AMIGA project represent 5 regions (Figure 4): Atlantic (Ireland, UK, Denmark, Netherlands), Boreal (Finland, Sweden), Continental (Austria, Germany, Slovakia), Mediterranean (France, Italy, Spain), and Balkans (Bulgaria, Romania). Four areas were selected based to a large degree on the Natura 2000 approach (Boreal, Atlantic, Continental, Mediterranean). In addition a fifth area (Balkans) was added, which includes two countries that according to Natura 2000 belong to four different zones. A research team from Argentina is also included in the consortium for activities relating to PMEM.

The choice of regional groups was driven by several factors. First of all, a desire to include the largest possible expected variability in environmental and agro-ecological conditions. Secondly it was considered the current availability of commercial GM crops as well as the possibility of deliberate release of GM crops for scientific purposes, which is quite variable among European countries. Finally, the selected range of
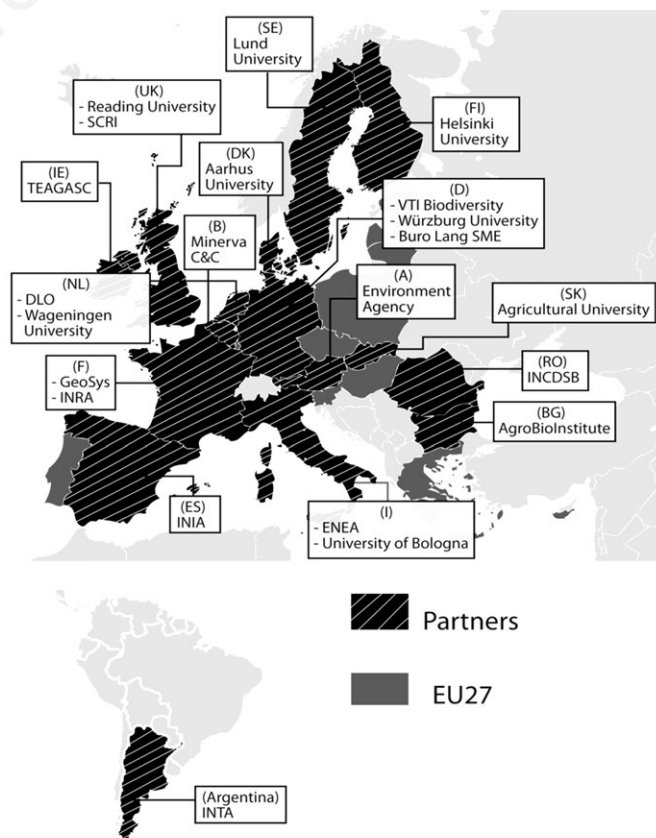

Figure 4. Partners of the AMIGA Consortium.

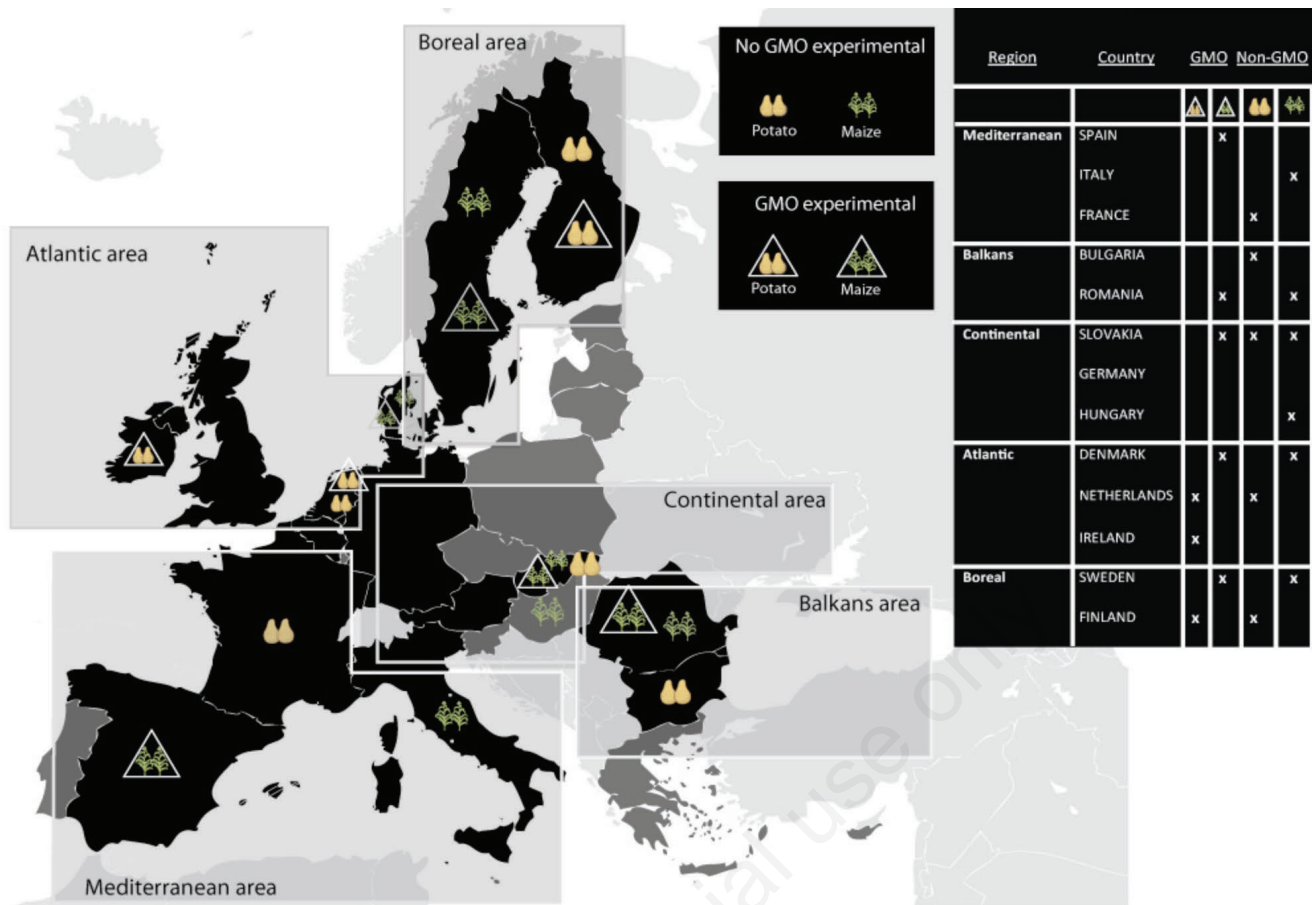

Figure 5. Map of Europe and of AMIGA countries in which field experiments with genetically modified crops (triangles) and surveys in conventional crops are being carried out for potato and maize.

regions enabled the incorporation of a range of different cropping practices which, according to EFSA (2010b), are important factors defining a receiving environment.

To support the above scientific studies, AMIGA is carrying out a range of laboratory, greenhouse and field studies. Maize and potato have been chosen as *model* crops in such studies because i) these two species are the only crops for which GM events have been approved for cultivation and ii) they cover a wide range of European receiving environment. Although AMIGA will use only these crops as case studies to develop innovative methodologies, such methodologies will be generic and transferable to assess the impacts of other GM events as well as other agricultural practices.

AMIGA project is not generating new information on the environmental safety of selected GM crops per se. Nor is AMIGA pre-developing any of the GMOs used but elaborating approaches and methods to reinforce risk assessment and monitoring procedures at the European level. An overview of the field experiments planned in the 5 geographical zones is given in Figure 5.

Conclusions

AMIGA has adopted a pan-European approach in order to bring together the relevant and necessary range of cross-disciplinary expertise in agronomy, molecular biology, genomics, soil biology, entomology, ecology, socio-economics and environmental impact assessment.

This ensures representative coverage of the main European agro-ecosystems and farming systems of Europe. Therefore, the impact of scientific and technological advances produced by AMIGA will not only benefit represented member states, but will be available for all member states.

Overall, AMIGA will foster a harmonized risk assessment approach in Member States for GM crops and will also contribute with novel methods and approaches to risk assessment for other pest control approaches.

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