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Applying an operating model for the environmental risk assessment in Italian Sites of Community Importance (SCI) of the European Commission Habitats Directive (92/43/EEC)

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Abstract

The fast development of agro-biotechnologies asks for a harmonized approach in risk analysis of GMO's releases, where "risk analysis" entails the assessment, management and communication of risk. Risk assessment consists in the evaluation of the likelihood that a hazard occurs, associated with the presence of the receptor(s) in the receiving environment, and the determination of their potential degree of exposure to the hazard. The whole process has to be both science based and applied case by case, and it involves a very complicated procedure that needs to be transformed in an operational and standardized tool. Beginning in 2003, an expert group on behalf of the Italian Ministry of the Environment elaborated an Operating Model for the Environmental Risk Assessment (OMERA) mainly addressed to scientists, companies, regulators, environmentalists, and also aiming to provide the stakeholders with a scientifically correct source of information. OMERA is based on a logic scheme, moving from the assumption that the occurring of a risk, associated to the release of a GMO into the environment, is strictly related to the presence of four elements: a) source; b) diffusion factors; c) dispersal routes; d) receptors. It comprises two main components: a conceptual model represented as a flowchart and an electronic Questionnaire (eQ) driven by a relational database (Microsoft Office Access). The eQ includes sets of questions specifically formulated for each box of the flowchart that create a "decision tree" that can be followed from the source to all the components of the conceptual model. Completing the eQ leads to the identification of the potentially affected receptors and related potential effects. Within the framework of a LIFE+ project (MAN-GMP-ITA) a multidisciplinary group was formed to validate and improve this methodology. The questionnaire has been filled simulating the release of herbicide tolerant GT73 oilseed rape and insect resistant MON810 maize in different experimental areas. The results attested that OMERA allows the collection and comparison of experimental data in a standard way and at the same time leads to the identification of receptors, potential effects and related monitoring activities specifically for the case study. The experiences described in this paper have shown the potential area of improvement of OMERA to achieve a more functional Decision Supporting System (DSS) that would give a quantitative risk evaluation and the extension to commercial fields. As for OMERA, the DSS will be user-friendly and could be easily modified and adapted to specific situations i.e risk assessment of GM crop field trials.

Key words: environment, risk assessment, environmental effects, GMO, plants, genetically modified, oilseed rape, maize, Italy.

Introduction

In the European Union, the deliberate release of Genetically Modified Organisms (GMOs) is regulated by Directive 2001/18/EC and Regulation (EC) No 1829/2003: [Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC] [Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (Text with EEA relevance)]. The Directive refers to the deliberate release into the environment of GMOs and sets out two regulatory regimes: Part B addresses the deliberate release of GMOs for any other purpose (including field trials), while Part C regulates the placing on the market of GMOs. The Regulation provides Community procedures for the authorization and supervision of GM food and feed, and additionally gives provisions for their labelling. While the Regulation's objectives are food and feed, it can also be applied to the authorization of GMOs for cultivation if they are intended for food or feed use. According to the mentioned Directive and Regulation, an applicant has to perform an Environmental Risk Assessment (ERA) to obtain authorization for the release of GMOs into the environment. The objectives and principles, together with the methodology, are outlined in Commission Decision 2002/623/EC: [Commission Decision of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (Text with EEA relevance)].

The ERA is defined as "the evaluation of risks to hu-

man health and the environment, whether direct or indirect, immediate or delayed, which the deliberate release or the placing on the market of GMOs may pose". The ERA is carried out on a case-by-case basis depending on the GM plant species, concerned trait(s), their intended use(s) and the characteristics of the receiving environment(s). The ERA is to be conducted in a scientifically sound and transparent manner based on available scientific and technical data, and on common methodologies for the identification and interpretation of relevant data. The ERA process is aimed to identify and evaluate potential adverse effects of GMOs, but also to provide the basis for subsequent monitoring plans together with risk management approaches.

According to the step-by-step ERA approaches developed worldwide for chemical or other environmental stressors (Hill and Sendashonga, 2003; Hill, 2005) the EU biosafety legislative framework foresees 6 steps (figure 1).

On November 2010 the European Food Safety Authority (EFSA) published its latest Guidance Document on the environmental risk assessment of genetically modified plants (EFSA GMO Panel, 2010). The Guidance Document represents a large effort to summarize and organize the operational procedures for conducting a comprehensive ERA on GM crops. This document discusses the six steps of ERA on Genetically Modified Plants (GMP), as reported in the Commission Decision 2002/623/EC, giving a comprehensive interpretation of each step and addressing seven specific areas of possible concern. The EFSA document represents updated guidelines on data requirements for ERA and includes several general cross-cutting considerations (e.g. choice of comparator/s, receiving environments, statistical approaches, long-term effects).

In the first step of ERA, which includes the Problem Formulation, the assessor has to scope out the problem by determining the main target of the assessment and the information needed (Yang *et al*., 2009; EFSA GMO Panel, 2010; Wolt *et al*., 2010) The starting point of this step is the characterization of hazards through a comparative safety assessment that would allow the identification of any biologically relevant differences between the GM plant and its conventional counterpart that may lead to harm. Subsequently, it is necessary to identify the exposure paths through which the GM plant may adversely affect the receiving environment. Finally, the identified potential adverse effects need to be linked to assessment endpoints (i.e., natural resources or ecological functions and ecosystem services representative of the Protection goals), in order to derive testable hypotheses allowing quantitative/qualitative evaluations.

According the European framework the risk (Risk characterization, step 4) is estimated combining the likelihood of exposure, (Exposure characterization, step 3) with the magnitude of associated effects (Hazard characterization, step 2). In the final step (overall Risk evaluation and conclusions), the overall risk covers each risk and the relevant management strategies (Risk management strategies, step 5).

The complex legislative process and the rapid development of agro-biotechnologies require harmonized and operative approaches in risk assessment of GMO's applications. At the Member State level an important contribution can be given by specific knowledge of the local receiving environment developed for supporting ERA.

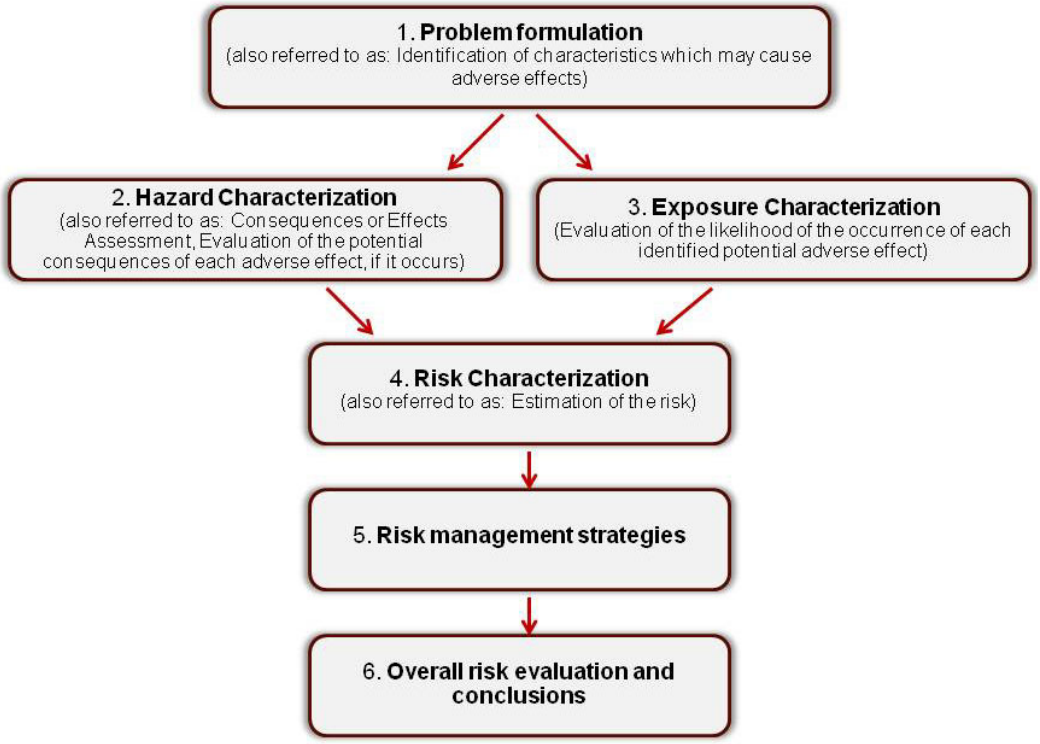

Figure 1. The six steps of the ERA according to EFSA Guidance on the environmental risk assessment of genetically modified plants (EFSA GMO PANEL. 2010).

Within the framework of a LIFE+ project named "Validation of risk management tools for genetically modified plants in protected and sensitive areas in Italy" (LIFE MAN-GMP-ITA) a multidisciplinary group was formed to validate and improve an existing ERA methodology (Sorlini *et al*., 2003) to perform ERA on GM plants, and to address the requirements of the European legislative framework on GMOs. Our goal was to develop an operative Decision Support System for Assessors and Managers involved in GMOs release evaluation.

The methodological proposal Operating Model for the Environmental Risk Assessment (hereinafter OMERA), is a tool to perform a risk assessment applied to GM crops field trials, that can be used by different assessors including applicant, competent authorities and other stakeholders. The system represents and describes the complex relationships between environmental receptors and the harmful characteristics of a GM crop in the field. Furthermore it leads to the identification of potential environmental effects on a case by case base.

The main aim of the present paper is to outline the logic of OMERA illustrating its keys features with the help of practical and concrete (real) examples applying it to different scenarios. In these scenarios the presence of GM oilseed rape and maize crops is simulated.

Materials and methods

OMERA

OMERA comprises two main components: a conceptual model represented as a flowchart (figure 2) and an electronic Questionnaire (eQ) driven by a relational database (Microsoft Office Access) (http://bch.minambiente.it/EN/Biosafety/propmet.asp). OMERA identifies questions to be answered to conduct a ERA process on a case by case base, it gives a list of potential impacts related to the case study. It allows the visualization of the potential pathways from a source of risk to potential impacts to the environment.

The flowchart represents the possible relations between a GM field trial and the receiving environment. It is based on the assumption that the occurrence of a risk is strictly related to the presence of four elements and of their interrelationships: Source; Diffusion Factors; Dispersal Routes; Receptors. The Source is where the organism is released and/or enabled to express its potential harmful characteristics. The Diffusion Factors are linked to the biological features of the GMP (e.g. pollen, seed). Dispersal Routes are the chemical, physical, and ecological characteristics of the receiving environment involved in the dispersal. Receptors are components of the ecosystem (including humans, animals, other plants, etc.) that may be affected by the Source.

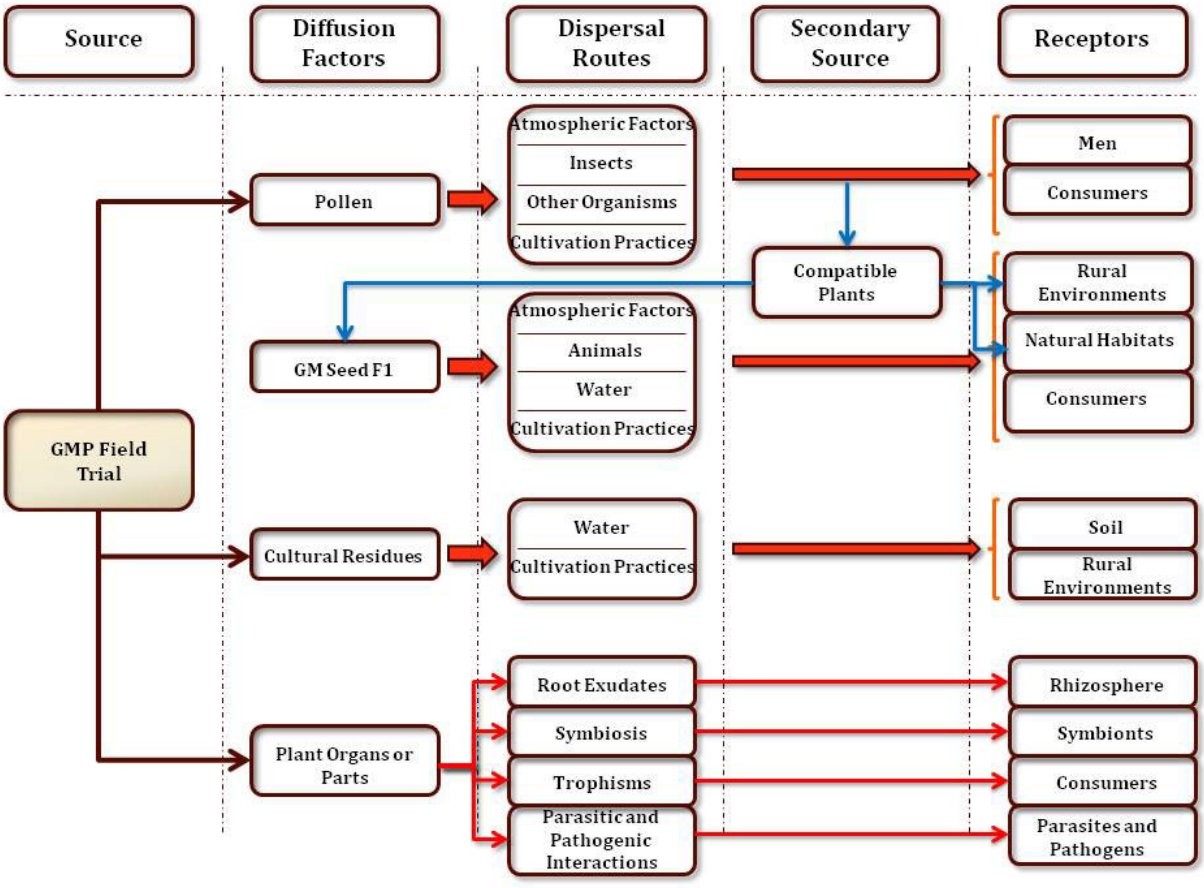

Figure 2. Conceptual model representing the potential relations between GMP in a field trial and the receiving environment.

The eQ includes sets of questions specifically formulated for each box of the flowchart that create a "decision tree" that can be followed from the source to all the components of the conceptual model. Completing the eQ leads to the identification of the potentially affected receptors and related potential effects. The eQ includes two kinds of question sets: the first group of questions is descriptive and they reflect the information required in the Annex III of the Directive 2001/18/EC. The second type of questions trigger a yes/no/don't know answer that can open or close specific paths. According to a precautionary approach, if a "don't know" answer is given, highlighting a lack of knowledge, the system automatically selects the path that represents the worst-case scenarios.

The first set of questions enables the characterization of the source. These questions consider:

- the biological characteristics: full name, biological form, leaves and stem, root system, fruit, seed, duration of vegetative cycle, infesting and invasive capacity; presence/absence of any toxins, and anti-nutrients and/or allergens present in the host plant, with the conventional counterpart taken as the baseline for each characteristic;
- the genetic modification: introduced or modified genes, transformation stability, expression of the insert, number of insert copies, insertion site, unintended inserted sequences, origin of inserted material;
- the conditions of the release: location of release site, farm size, release area size, size of area cultivated with GMP, release duration, release period, cultivation practices.

Once the characterization of the source has been completed, different sets of key multiple choice questions (yes, no, don't know) can activate or block specific DFs: pollen, seed, crop residues, plant organs or parts (figure 3). The next step is the characterization of the activated DFs where the descriptive set of questions refers to the biological attributes of the crop and the environmental factors influencing them. Field management practices are taken into account in this step as well. These questions are useful for exposure characterization.

For each activated DFs specific DRs are identified and described, including those acting on the exposure level and mitigation measures DRs (figure 2); include atmospheric factors, insects or other animals, agricultural practices, etc., and represent the routes that lead DFs to specific receptors.

Once the DFs and DR has been assessed, the questionnaire seeks data on the presence or absence of related receptors at reachable distances and of harmful new characteristics in the GMP DFs.

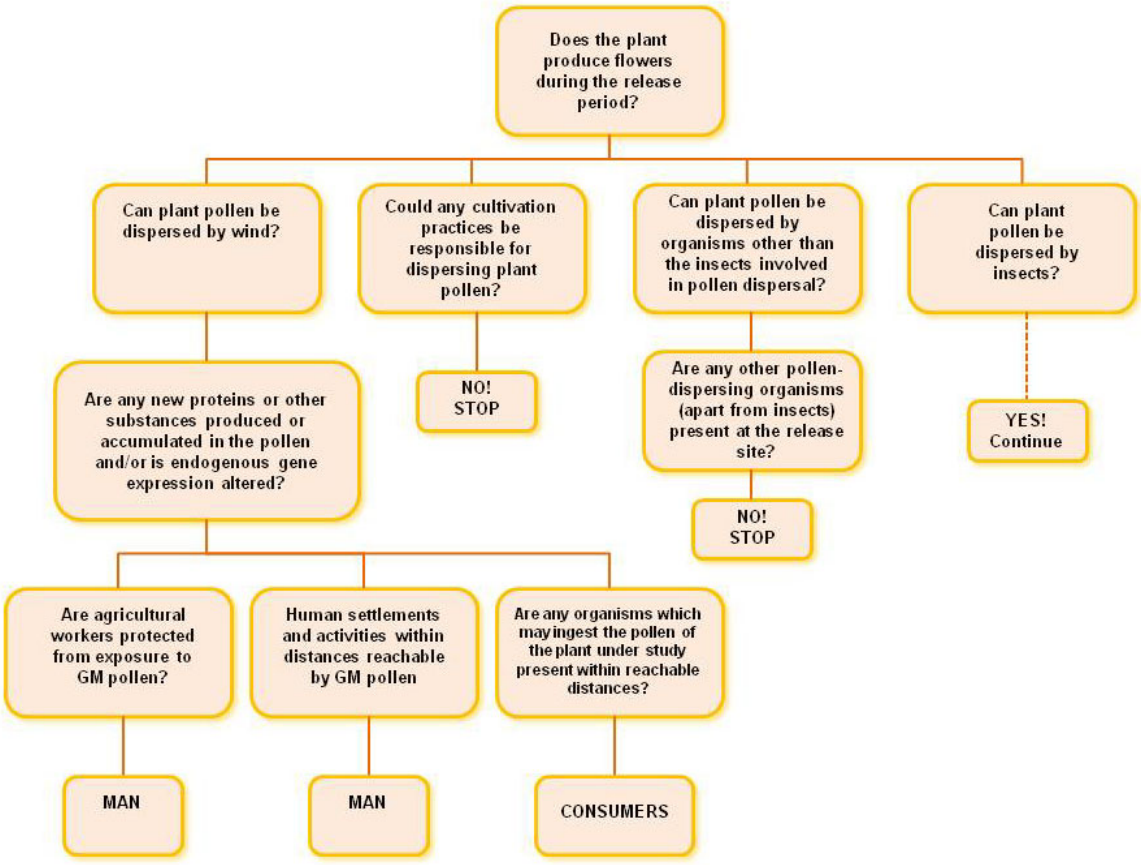

Figure 3. Flowchart resuming an example of key multiple responses questions activating the pathways related to the DF Pollen in the analyzed oilseed rape case studies. In this specific example the activated Dispersal Routes are Atmospheric Factors and Insects.

Hybridization between the crop plants and sexually compatible plants near the field can produce viable seeds that if dispersed into the environment could lead to potential indirect effects. For this reason, the receptor Compatible Plants is considered a Secondary Source of risk. This source is split into three sub-groups: GM crops, non-GM crops, and wild sexually compatible species. GM crops are maintained in a separate group because the cross-fertilization with different GM events would lead to specific effects related to the occurrence of stacked genes.

Questionnaire responses

To test the process, the questionnaire was completed independently by four different groups of experts, one for region, from the consortium of the Life+ project. It was compiled simulating the presence in the field of herbicide tolerant GT73 oilseed rape and insect resistant MON810 maize. Both these events are already authorized under Directive 18/2001/EC, thus most of the information requested in the questionnaire related to the molecular and botanical characteristics of the crops is available in the EU register of GM products (http://ec.europa.eu/food/dyna/gm_register/index_en.cfm) established in accordance with the Regulation (EC) 1829/2003 and in related documents like the Summary Notification Information Formats (SNIFs), the EFSA opinions, and the Consensus documents edited by OECD (OECD, 1997, 2001, 2003, 2007). Bibliographic sources were also used to complement field observations, in particular those on climatic and soil characteristics, and functional biodiversity.

The eQ has been applied in five Sites of Community Importance (SCI) from the European Commission Habitats Directive (92/43/EEC) in Italy: two in Emilia Romagna, one in Lazio, one in Basilicata and one in Sicily. Non-GM fields of oilseed rape and maize were cultivated in the selected areas (table 1) to provide details about the crop-environment interactions at each location.

Field trials

Oilseed rape fields

The same experimental layout was prepared in three different locations during the 2011 growing season: SCI IT4050001 (Gessi bolognesi e Calanchi dell'Abbadessa), SCI IT6030015 (Macchia di Sant'Angelo Romano), SCI IT9220090 (Costa Ionica and Foce Bradano). The experimental designs of the three sites were arranged for both short-distance (1-20 m) and long-distance pollen (50-500 m) dispersal studies, as defined in the literature (Scheffler *et al*., 1993; 1995; Funk *et al*., 2006). The oilseed rape (*Brassica napus* L.) varieties used in the field trials were the free-pollinating "Ceres" and "Dante" cultivars, respectively selected as donor and receiving crops, where the donor simulates the GM crop. The donor plot was surrounded by eight receptor plots.

Table 1. Sites of Community Importance, area description.

Region / Name	Surface (ha)	EU protection status	Short description	Crop
Apulia Basilicata / Costa Ionica Foce Bradano	473	pSCI NATURA 2000 Code: IT9220090	The area is a humid coastal area rich of different habitat types, it is particularly vulnerable due to the coastal erosion and the peculiar climatic conditions. It is therefore very important the monitoring of any human activity for its potential effect on the area. The area of the SCI is for 50% public property and 50% private. Main land uses are agriculture (30%), touristic activities (20%), urban (5%) and conservation (45%)	Maize, oilseed rape
Emilia-Romagna / Bentivoglio, S. Pietro in Casale, Malalbergo e Baricella	3224	SPA and pSCI NATURA 2000 Code: IT4050024	The fragmented site comprises rural areas and is in particular is surrounded by arable crops, it is is 100% private property, including 5% of channels. Three areas of community interest cover about 14% of the site: - natural lakes with community plants of *Magnopotamion* or *Hydrocharition*; margin rivers with plant communities of *Chenopodion rubri* and *Bidention*; and forest of *Salix alba* and *Populus alba*	Maize
Emilia-Romagna / Gessi Bolognesi, Calanchi dell'Abbadessa	3965	pSCI NATURA 2000 Code: IT4050001	The crops are common on most of site (arable crops, vegetable crops, fruit orchards). The area is for 90% of private ownership and 10% of public ownership (Parco dei Gessi). The woods are present on 14% of the site. The grass-lands cover about 25% of the area. The site is included (86%) within the "Regional Park of Gessi Bolognesi and Calanchi dell'Abbadessa". Eleven habitat included within the Habitats Directive cover about 45% of the area	Oilseed rape
Lazio / Macchia di S. Angelo Romano	798	pSCI X NATURA 2000 Code: IT6030015	The site has an important role for many species of plants and animals of particular naturalistic value. Macchia di S. Angelo Romano is an important halting area for several migratory birds of Community Interest. The SCI area is not particularly vulnerable but the monitoring of any human activity is important. Main land uses are agriculture (15%), urban and industrial (5%), woods (30%), water bodies (5%) and conservation (45%)	Maize, oilseed rape
Sicily / Raffo Rosso, M. Cuccio e Vallone Sagana	6098	SIC ZPS NATURA 2000 Code: ITA020023	The area includes mountains as well coastal agricultural and urban areas. The site has an important role for many species of plants and animals of particular naturalistic value and endemic species. The site includes habitat from which several species where used for their characterization. Main land uses are grassland and steppes (70%), heath (15%) and woods (5%) but also agriculture and tourist activities	Maize

Maize fields

For the maize field, four different locations were planted during the 2011 growing season: in Emilia, the maize field was approximately 6 hectares and located near SCI IT4050024 - Biotopi e Ripristini Ambientali di Bentivoglio, San Pietro in Casale, Malalbergo e Baricella. In Lazio the field study, approximately two hectares, was carried out at the experimental farm of the Agriculture Research Centre at about two kilometers of the protected area SCI IT6030015 (Macchia di Sant'Angelo Romano). In Basilicata the research activity was conducted on a maize field of approximately one hectare, at the experimental farm "Pantanello" located approximately one kilometre from the protected site SCI IT9220090 (Costa Ionica - Foce Bradano). In Sicily the maize field was approximately 0.8 ha at the experimental farm and located less than one kilometre the protected site SCI ITA020023 (Raffo Rosso, Monte Cuccio e Vallone Sagana).

Results

The five study areas are representative of the potential Italian "receiving environment" for the chosen GMP species, in terms of environmental conditions, cropping systems, *biocoenosis*, and relevance of surrounding habitats. At least one field of the two crops (oilseed rape and maize) was grown at each site, except for the site in Sicily where only maize was grown. Thus seven different risk scenarios have been analyzed and seven diverse questionnaires have been compiled.

In the ERA procedures, the characteristics or biology of the parental plant are important sources of information because knowledge of the biology of the comparator plant provides baseline information suggestive of the likely behaviour of the transgenic plant in the environment into which it is introduced. The first section of the eQ questions corresponds to a comparative analysis to establish if the GMPs show the same characteristics as the parental line used as comparator. The questions identify the botanical and agronomic characteristics of the GMP, together with the molecular characterization of the inserted genes and the insertion site in order to identify intended and potential unintended effects.

The transgenes considered in this study are integrated as a single copy in the plant genome. In oilseed rape GT73, the GOXv247 and CP4 EPSPS proteins are constitutively expressed, both conferring tolerance to glyphosate-based herbicides; while in maize MON810 the CRY1Ab insecticidal protein is constitutively expressed. Filling in the questionnaires using data and information presented in EFSA opinions for oilseed rape (EFSA GMO Panel, 2004) and maize (EFSA GMO Panel, 2009), no unintended effects linked to the new molecular characteristics have been reported.

Pathway for pollen and seed DFs

Figures 4 and 5 show the activated pathways for the oilseed rape and maize field trials related to pollen and seed DFs and corresponding receptors. In all cases pollen and seeds will be produced and dispersed, but different receptors are present.

Reviewing the questionnaire responses, the oilseed rape pollen will be produced but according to the EFSA opinion (EFSA GMO Panel, 2004) no allergenic or toxic compounds are expressed in pollen grains, thus the pollen route to human and other consumers' receptors is closed. By contrast, the pathway leading to compatible plants is activated. The presence of wild compatible species in all the three sites has been observed and, according to the experimental design, cultivated oilseed rape is present at distances reachable by pollen; for these reasons potential effects on the rural environments and natural habitats receptors through specific interbreeding can be hypothesized. In this case risks should be evaluated considering frequency of the gene flow and the ecological role of the resulting offspring.

The system then follows the fate of viable F1 seeds produced in the GM field; as shown in figures 4 and 5 they can be dispersed by diverse DRs reaching rural and natural environment receptors. However considering the GM oilseed rape phenotype, potential effects have been hypothesized only in rural environments. Indeed oilseed rape is considered a weed species but not invasive (OECD, 1997); the herbicide resistance characteristic conferred by the transgenes would cause enhanced persistence in field and field margin of the resulting offspring.

Compared to the oilseed rape cases, the maize case studies present important differences in activated pathways (figures 4 and 5), depending on botanical and ecological characteristics of the crop and on the characteristic of the inserted transgene. As a Bt maize release has been hypothesized, the presence of toxin Cry1Ab in pollen grains actives the pathway leading to the receptor consumers, which would include both target and non-target Lepidoptera feeding pollen maize.

The resulting potential effects identified drive the system to ask for the characterization of the target and non-target Lepidoptera populations present in the sites during the GM release. The Secondary Source Compatible Plants pathway is not activated, for either cultivated or wild plants. Indeed, at distances reachable by the pollen, maize crops are not cultivated and wild relatives of maize crop are not present in Europe. As a result no potential effects on rural environment and natural habitats due to vertical gene flow can be identified.

F1 seeds could be produced only within the experimental fields. Due to the specific agricultural conditions within the three maize case studies, some differences in the DF F1 Seed are reported. In fact, in Basilicata field trial maize is harvested before seeds mature, thus the pathway that leads to DF Seed is blocked. Where F1 seeds are produced, no effects can be hypothesized for the natural and rural environment receptors, because maize is neither invasive nor weedy (OECD, 2003). In addition, maize cannot survive outside the field in Italian environmental conditions. The CRY toxin is present in seed too, but the presence of CRY-sensitive organisms feeding on maize seeds as a major food source was considered unlikely, hence the pathway to the receptor consumers of seed is closed.

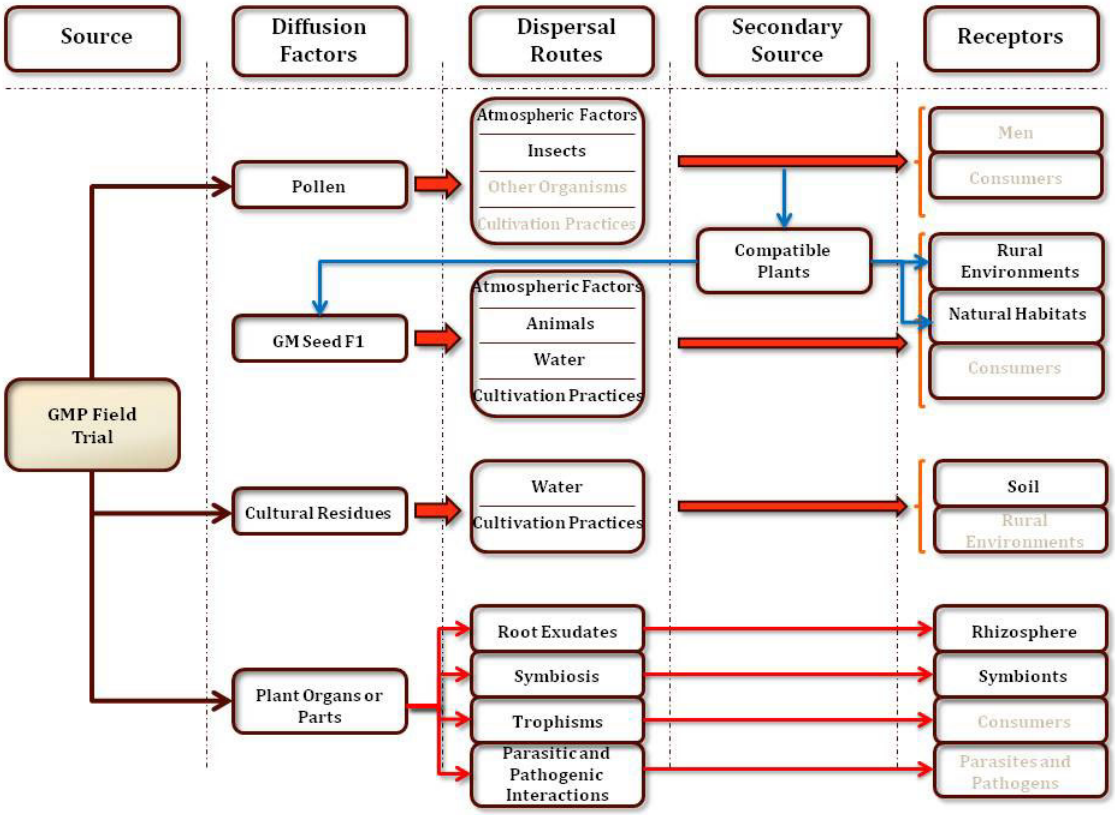

Figure 4. Flowchart showing the activated pathways in relation to the three oilseed rape field trials. The non activated items are transparent.

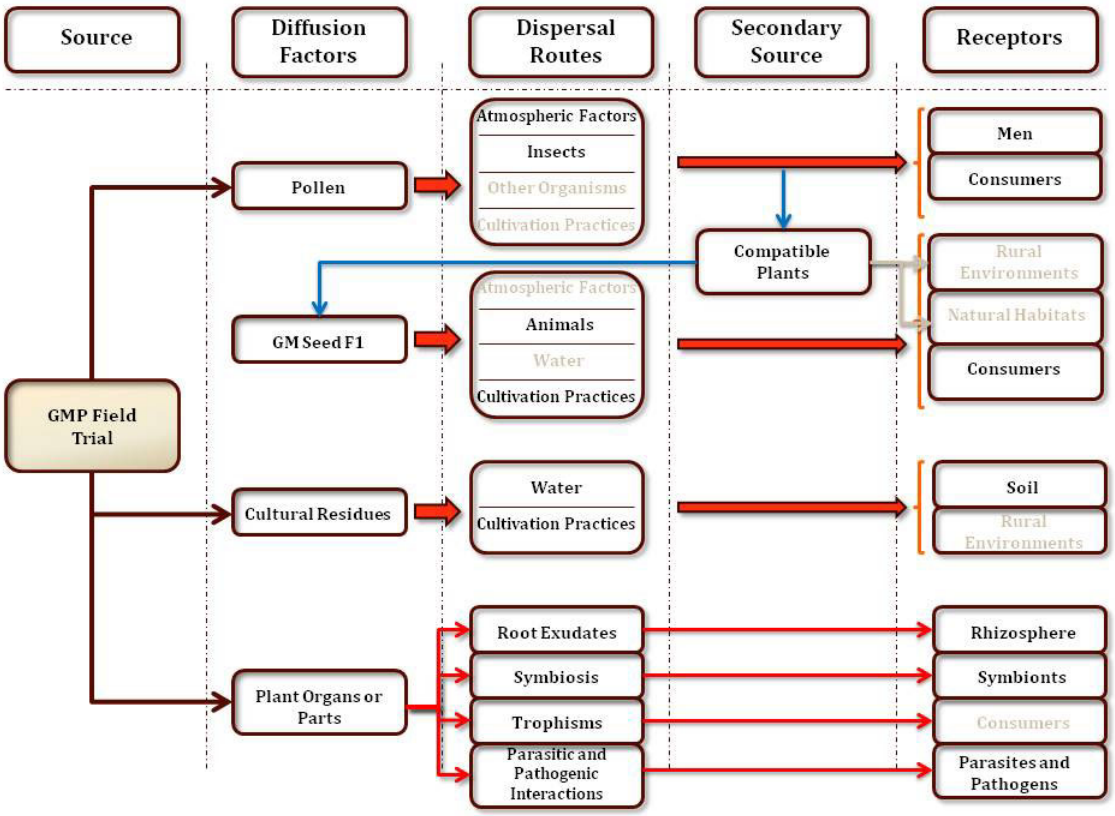

Figure 5. Flowchart showing the activated pathways for maize case studies. The non activated items are represented by dotted lines or transparent.

Pathway for cultural residues and plant organs DFs

Even if oilseed rape and Maize case studies show the same results (figures 4 and 5)**,** these results are obtained by different kinds of responses. In fact, where for the maize case the diverse eQ pathways are activated by affirmative answers, for the oilseed rape case the experts have given several "don't know" responses. For example, to the question, "Are there any new substances or proteins in the GMP root exudates?" in the maize case the answer was "Yes," as reported in diverse studies on maize MON810 where Bt toxin in root exudates has been identified (Saxena *et al*., 2004). In the oilseed rape case root exudates, also in relation to the inserted gene product, have not yet been investigated, consequently the answer in questionnaire was "don't know."

As described above, the software considers this lack of knowledge as a source of risk and opens the potential pathways representing the worst case scenario and therefore resulting in the same list of potential effects (tables 2 and 3). Monitoring actions should be set up to verify the occurrence of the hypothesized effects. In maize case diverse potential effects on non-target organism populations have already been studied, but only in laboratory and microcosm studies, representing a worst scenario, some effects have been identified (Zwahlen *et al*., 2003; Brusetti *et al*., 2004; Castaldini *et al*., 2005; Kramarz *et al*., 2009), while in other researches no one has been reported (Saxena and Stotzky, 2001a; 2001b; Griffiths *et al*., 2006; Vercesi *et al*., 2006; de Vaufleury *et al*., 2007; Verbruggen *et al*., 2012). For oilseed rape tolerant to herbicides no specific studies have been performed yet.

Identified effects

The complete list of the identified potential effects for each receptor for oilseed rape and maize case studies is shown respectively in tables 2 and 3. As indicated in the tables, some of the effects are direct results of the interaction between DFs and Receptors, while others depend on the consequences of this interaction. It is important

Table 2. List of the Identified Potential effects (column 1) *versus* receptor (row 1) for the three case studies of oilseed rape. The activated Diffusion factors are specified together with the distinction in direct (D) and indirect effect (I).

	Natural habitats	Rural environment	Rizosphere and symbionts	Soil
Potential changes to agricultural practice		Pollen (I) Seed (I)		
Potential changes to agrobiodiversity		Pollen (I) Seed (I)		
Potential changes to biodiversity	Pollen (I)			
Potential changes to edaphic fauna biodiversity				Pollen (I) Seed (I) Residues (D)
Potential changes to GMP development			Plant (I)	
Potential changes to GMP productivity			Plant (I)	
Potential changes to rhizosphere abiotic component			Plant (I)	
Potential changes to soil fertility				Pollen (I) Seed (I) Residues (I)
Potential changes to soil microbe and fungal biodiversity				Pollen (I) Seed (I) Residues (D)
Potential changes to structure of microbial and fungal rhizosphere populations			Plant (D)	
Potential changes to structure of non-symbiotic populations			Plant (D)	
Potential changes to structure of rhizosphere populations			Plant (D)	
Potential changes to structure of symbiotic populations			Plant (D)	
Potential colonisation of natural habitats	Pollen (D)			
Potential food chain contamination		Pollen (D) Seed (I)		
Potential increase of weeds		Seed (D)		
Potential pollution of natural genetic resources	Pollen (I)			
Potential uncontrolled GMP presence in the environment	Pollen (I) Seed (D)			

Table 3. List of the Identified Potential effects (column 1) *versus* receptor (row 1) for the four case studies of maize. The activated Diffusion factors are specified together with the distinction in direct (D) and indirect effect (I).

	Consumers	Men	Plant pathogens	Rizosphere and symbionts	Soil
Potential allergenic effects on population		Pollen (D)			
Potential allergenic effects on workers		Pollen (D)			
Potential changes to edaphic fauna biodiversity					Residues (D)
Potential changes to GMP development				Plant (I)	
Potential changes to GMP productivity				Plant (I)	
Potential changes to rhizosphere abiotic component				Plant (I)	
Potential changes to soil fertility					Residues (D)
Potential changes to soil microbe and fungal biodiversity					Residues (D)
Potential changes to structure of microbial and fungal rhizosphere populations				Plant (D)	
Potential changes to structure of non-symbiotic populations				Plant (D)	
Potential changes to structure of rhizosphere populations				Plant (D)	
Potential changes to structure of symbiotic populations				Plant (D)	
Potential changes to target pathogen host range			Plant (I)		
Potential development of resistant target pathogen populations			Plant (I)		
Potential effects on biodiversity	Pollen (I)				
Toxicity potential for consumers of new substances in pollen	Pollen (D)				

to note that potential effects are not risks; they should be evaluated for their probability and consequences in order to complete the risk assessment.

The same effect can occur via different DFs (tables 2 and 3). In the oilseed rape case studies the indirect potential changes to agricultural practice on the rural environment receptor is due both to pollen and seed DFs. Again, on the same receptor, the potential food chain contamination derives from pollen and seed dispersal, but in this case the first is a direct effect, while the second one is indirect. Indeed, the pollen produced by the GM field can pollinate the neighbouring compatible fields, while oilseed rape seeds can be dispersed into the rural environment by animals, wind, or other vectors, becoming weeds in cultivated fields and potentially contaminating oilseed rape crops in the following seasons.

Similarly, for the soil receptor some of the identified potential effects, such as the potential changes to soil fertility, are consequences of other effects: see the potential changes to soil microbe and fungal biodiversity and the potential changes to agricultural practices. Comparing the lists of the identified effects in the questionnaires, a unique difference can be identified only in one of the oilseed rape case studies. It can be observed for the rural environment receptor in relation to seed dispersal; this result is due to a specific rural environmental characteristic in the Basilicata region where oilseed rape is not normally cultivated and thus no cultivated oilseed crops are present.

The identified potential effects related to the soil receptor are the same in all the case studies. Potential effects do not depend on soil characteristics, but are essentially due to lack of information on changes in bromatological composition of plant residues. The occurrence of effects can be assessed with targeted monitoring activities.

Discussion and conclusion

Environmental risk assessment of GM crops is performed to evaluate whether any harm might arise from the deliberate release of a genetically modified plant into the environment. It is generally based on information on the characteristics or biology of the unmodified parental plant, the trait(s) engineered and the resulting phenotype, the characteristics of the receiving environment, the intended use, and the interaction among these factors (EFSA GMO Panel, 2010).

The ERA, within the framework of the relevant legislation, establishes the context for identification of:

- potential hazards that might be associated with the transgenic plant;
- aspects of the receiving environment that might be harmed by the identified potential hazard;

- the attributes of the receiving environment that may need protection;
- pathways by which the identified potential hazard might move beyond the environment of cultivation;
- information, from existing literature or new studies, needed to evaluate the probability that the hazard might exert an effect and that harm might occur and to evaluate the magnitude of any such harm;
- targeted risk management strategies based on the outcome of the risk characterization.

OMERA has been confirmed as a useful tool performing the different steps of ERA. The implementation of OMERA gives a list of potential effects and related receptors, allows the visualization of the pathways starting from the source, going through dispersal routes and diffusion factors, and reaching the potential receptors. It also helps to identify data and information necessary to evaluate the likelihood of the identified effects. It provides a clear picture of the relation between the source of harm and receptors, giving assessors an easy and unequivocal opportunity of carrying out causes and effects analyses. In addition, the visual representation will allow the identification of hot spots that should be considered during risk management procedures, using the diagrams represented in figures 4 and 5, and would help in identifying points where containment and management measures could be adopted. For example, the presence of maize pollen in a small field trial could be managed by detasselling the flowers or increasing the distance from compatible crops to avoid crop contamination. In the case of oilseed rape, to reduce the probability of the occurrence of the identified potential increase of weeds, crop rotation practices could be applied together with the use of an alternative, non-glyphosate herbicide. In case of field trials, monitoring activity and manual uprooting could be another option.

It is useful to stress that the proposed model can be applied both *ex ante* and *ex post* release: in the *ex ante* phase to identify potential effects, in the *ex post* release phase to select risk management procedures, to set up monitoring activities, and to verify the success of the risk management procedures applied.

OMERA can be considered a Decision Support System not only for the elaboration of risk hypotheses and the establishment of monitoring programs, but also for the selection of the most effective management strategies. Due to its characteristics it can be used by different of risk assessors, competent authorities, organisations. As further auxiliary application, OMERA could be considered as training tool for beginners assessors, also to elicit their opinion.

The experiences described in this paper have shown one potential area of improvement of OMERA to achieve a more functional DSS. Most of the questions related to botanical and agronomical characteristics are already well established in official documents (i.e. OECD publications "Series on Harmonization of Regulatory Oversight in Biotechnology": Consensus Documents on the biology of etc.) and this information is necessary for any ERA. Given this initial assumption, it is clear that this kind of information could be gathered in a database as a whole file that would automatically be used in the DSS. The same procedure could be adopted for well-known transgenes. In this way the user would only be asked to complete the questions related to the case specific characteristics such as those relating to the site, including soil and climatic characteristics.

A standardized procedure in performing ERA and in collecting data would improve information sharing and risk communication on GM plants for all stakeholders, including the general public. This method will lead to a common risk assessment, where all the relevant system components are taken into account. The structure and contents of the methodology can be easily adapted and implemented to different situations. New questions can be edited to focus on a specific receptor, for example insects to be used as bio-indicators; or to stress a particular topic (e.g. bioremediation). At the same time the conceptual model can be developed starting from different sources of harm (e.g. transgenic insects or microorganisms).

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