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AN OPERATING MODEL FOR THE ENVIRONMENTAL RISK ASSESSMENT APPLIED TO ITALIAN SITES OF COMMUNITY IMPORTANCE: IDENTIFICATION OF POTENTIAL EFFECTS ON SOIL

UN MODÈLE DE FONCTIONNEMENT POUR L'ÉVALUATION DES RISQUES ENVIRONNEMENTAUX APPLIQUÉ AUX SITES ITALIENS D'IMPORTANCE COMMUNAUTAIRE: IDENTIFICATION DES EFFETS POTENTIELS SUR SOL

UN MODELLO OPERATIVO PER LA VALUTAZIONE DEL RISCHIO AMBIENTALE APPLICATA AI SITI ITALIANI DI IMPORTANZA COMUNITARIA: IDENTIFICAZIONE DEI POTENZIALI EFFETTI SUL SUOLO

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Abstract

The fast development of agro-biotechnologies asks for a harmonized approach in risk analysis of GMOs releases. An Italian experts group has elaborated an operating model for the environmental risk assessment (OMERA) based on the assumption that the occurring of a risk is related to the presence of four components: source, diffusion factors, dispersal routes, receptors. This model has been further developed to become a Decision Supporting System based on Fuzzy logic (FDSS) to assessors and notifiers. It is a web based Questionnaire that conducts the user through a decision tree from the source to the receptors and leads to the identification and assessment of the risks. The FDSS has been tested on case studies, simulating, as source, herbicide tolerant oilseed rape and insect resistant maize. The resulting identified potential effects on soil are changes to structure and microbial diversity.

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

Résumé

Le développement rapide des agro-biotechnologies demande une approche harmonisée en matière d'analyse des risques des GMO. Un groupe d'experts italien a élaboré un modèle opérationnel pour l'évaluation des risques pour l'environnement (OMERA), basé sur l'hypothèse que le survenant d'un risque est lié à la présence de quatre éléments: la source, les facteurs de diffusion, les voies de dispersion, les récepteurs. Ce modèle a été développé pour devenir une véritable système d'informa-

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tion décisionnel sur la base de la logique fuzzy (SIDF). Un questionnaire Web mène l'utilisateur à travers un arbre de décision à partir de la source aux récepteurs et conduit à l'identification et l'évaluation des risques. Le SID a été testé en simulant, en tant que source, colza tolérant à un herbicide et maïs résistant aux insectes. Pour le sol, les effets potentiels identifiés sont les changements à la biodiversité et aux pratiques agricoles.
Mots clés: *environnement, évaluation des risques, effets environnementaux des OGM, plantes, sol, génétiquement modifié, colza, maïs, Italie.*

Riassunto

Il rapido sviluppo delle agrobiotecnologie richiede un approccio armonizzato nell'analisi del rischio dei rilasci di OGM. Un gruppo di esperti italiani ha elaborato un modello operativo per la valutazione dei rischi ambientali (OMERA), basato sul presupposto che l'insorgere di un rischio è legato alla presenza di quattro componenti: fonte, fattori di diffusione, vie di migrazione, recettori.. Questo modello è stato ulteriormente sviluppato fino a diventare Sistema di Supporto alle Decisioni basato sulla logica fuzzy (FDSS) per valutatori del rischio e notificanti. Un questionario web-based conduce l'utente attraverso un albero decisionale dalla sorgente ai ricettori e ha porta alla individuazione e valutazione dei rischi. . Il DSS è stato testato su casi-studio usando come fonti colza tollerante agli erbicidi e mais resistente agli insetti. I potenziali effetti identificati per il suolo sono i cambiamenti nella struttura e nella diversità della comunità microbica.
Parole chiave: *ambiente, valutazione del rischio, effetti ambientali, OGM, piante, suolo, geneticamente modificato, colza, mais, Italia.*

Introduction

In European Union, the release into the environment of GMOs is regulated by Directive 2001/18/EC, (*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*), implemented in Italy by Legislative Decree 224/2003 (*Legislative Decree of 8 July 2003, n. 224, implementing Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. Posted in: Official Journal of General Series n. 194 of 22 August 2003, ordinary supplement no. 138*). The legislation provides for two different authorization procedures according to the use required, the one for experimental purposes (Title II) run at a national level, and the one for commercial purposes (Title III) managed at Community level. Regarding the placing on the market in EU is also in force Regulation (EC) 1829/2003 (*Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed*), which currently represents the main reference legislation and which is applicable to GMOs intended for food&feed uses. Authorization, unless special provisions, is valid throughout the EU for a period of 10 years (Lener and Staiano 2010).

According to the Directive and the Regulation, an applicant has to perform an Environmental Risk Assessment (ERA) to obtain the authorization for the release of GMOs into the environment. The Annex II (*Annex II of the Directive has been integrated by the European Commission Decision 2002/623/EC 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. Published in: Official Journal of the European Union L 200 of 30 July 2002*) of Directive 2001/18/EC establish in broad terms the objective to be achieved, the elements to be considered and the principles and methods to be followed to carry out the environmental risk assessment (ERA). The ERA is formally defined by the Directive as "*the evaluation of risks to human 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*". ERA should be carried out case by case, meaning that its conclusion may depends on the GM plants and trait concerned, their intended uses and the potential receiving environments. The ERA process should lead to the identification and evaluation of potential adverse effects of the GMO, and it should be conducted also for identifying if there is a need for risk management and a specific monitoring plan. ERA process is often performed in presence of incomplete and imprecise data. In 2010, EFSA (*European Food Safety Authority (EFSA) established by Regulation (EC) No 178/2002*) has published new guidelines for the environmental risk assessment of GM plants (EFSA GMO Panel, 2010), which provide a summary of the different interpretations and examine in great detail the main areas of risk. However, at present there are no universally accepted instruments to support the execution of the ERA for GMOs.

The aim of this work is to describe the application of standardized tools developed specifically for the ERA of GMO field trials. These methodologies have been applied to the case studies simulating the environmental release of two GM crops, herbicide tolerant oilseed rape and insect resistant maize, analyzed during a Life+ project (Man-Gmp-Ita) which took place from 2010 to 2013. In this work we will present the results obtained on case-studies in general, and on the soil component in more details.

Materials and methods

Case studies

The case studies have been analyzed within a Life+ project (Man-Gmp-Ita, http://www.man-gmp-ita.sinanet.isprambiente.it/progetto), where it was assumed the release of herbicides tolerant GM oilseed rape (*The oilseed rape GT73 is produced by Monsanto and obtained with the technique of transformation mediated by Agrobacterium tumefaciens. In the genome of rape GT73 were inserted cp4epsps and gox genes: the first gene expresses a variant of the enzyme EPSPS that confers tolerance to glyphosate, the second one expresses the GOX enzyme that confers tolerance to glyphosate too. This product has been authorized in the EU for use in food as an existing product in accordance with Art. 8 (1) (b) of Regulation (EC) No 1829/2003, and in feed with the Decision 2005/635/EC*

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concerning the placing on the market of products of oilseed rape (Brassica napus L., GT73 line) genetically modified for tolerance to the glyphosate herbicide) and insect resistant GM maize (MON810 - The MON810 maize is produced by Monsanto and obtained by transforming the genome of maize with the biolistic technique. In the maize genome has been introduced *cry1Ab* gene arising from *Bacillus thuringiensis*, and MON810 expresses the Cry1Ab protein, a toxin for Lepidoptera, which gives to the plant the ability to resist to the attacks of harmful Lepidoptera. The product has been authorized in the EU for use in food and feed and for cultivation by Decision 98/294/EC concerning the placing on the market of genetically modified maize (*Zea mays* L., MON810 line), according to Directive 90/220/EEC, and it is in the process of renewal of authorization for the same uses (http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=11) even if we didn't utilize the GM crops but the conventional counterpart (Lener *et al.* 2013). As hypothetical GM release sites, five areas nearby Italian SCIs (Sites of Community Interest) have been used but in this paper we report only the results of the Lazio areas. The Lazio field trials have been conducted in the season 2010 and 2011 inside the Research farm "Tor Mancina" of the CRA. This area is largely used for cultivation and the main natural environment is constituted by riparian vegetation (see Figure 1).

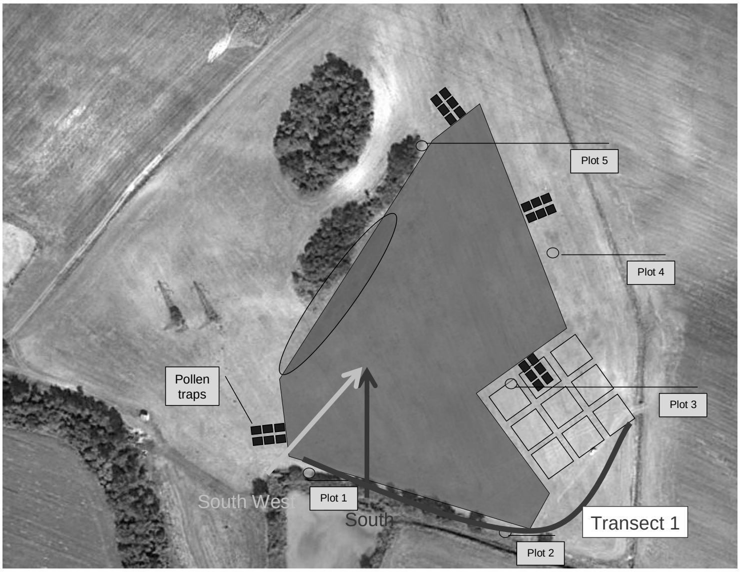

Figure 1

The experimental field area of 2011 season. The linear transect 1 for non-target insects sampling, conducted in 2011 and 2012 season, and is along maize field margins.

Risk analysis

The environmental risk analysis has been conducted applying two different on line free tools: OMERA (http://bch.minambiente.it/IT/Biosafety/propmet.asp) and Fuzzy Decision Support System (FDSS) (http://www.man-gmp-ita.sinanet.isprambiente.it/progetto).

The first one, developed by an expert group of the Italian Ministry of Environment (Sorlini et al. 2005), is a methodology consisting of two main components: a conceptual model represented as a flow chart (see Figure 2) and an electronic questionnaire (eQ) driven by a relational database management system (developed with Microsoft Office Access).
The FDSS apply the fuzzy logic at the modified OMERA, giving the opportunity to obtain a quantitative assessment of the identified risks (Camastra *et al.* 2014).

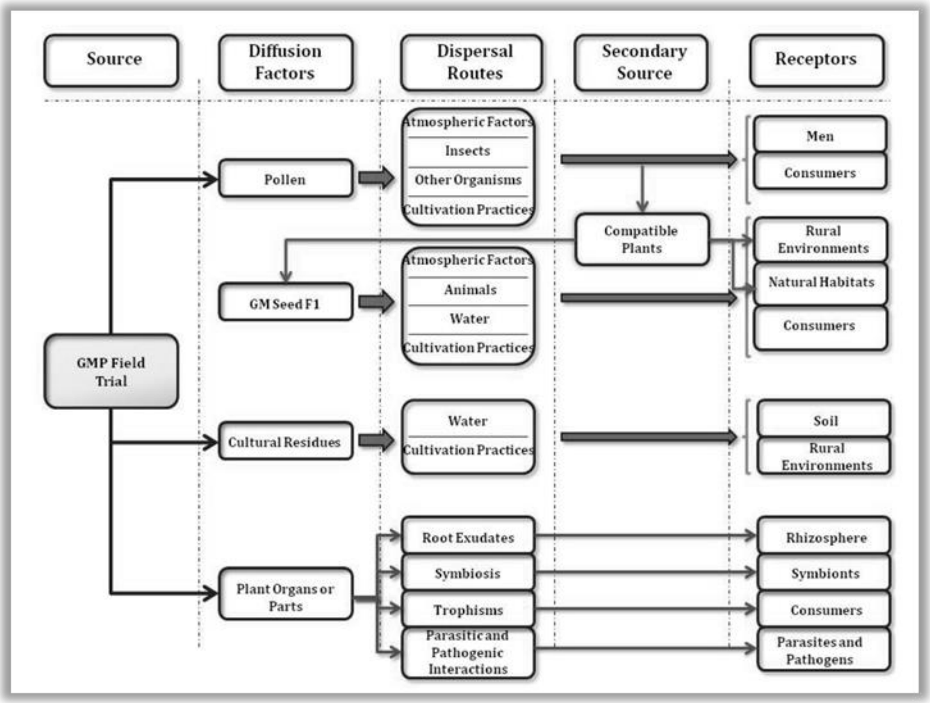

Figure 2 - *Conceptual model representing the possible paths from a specific source to a given receptor through the diffusion factors and the dispersal routes.*

Results

Application of OMERA to the Lazio case studies, leads to the identifications of the potential affected receptors and the related potential effects (Table 1); considering that the same effects could arise from different diffusion factors the latter are indicated in the table. Main differences listed between maize and oilseed rape (OSR) depend on crop botanical and agronomic characteristic and from the inserted genes and related products. Principally for maize MON810 the presence of toxins in pollen and plant tissues drives potential effects to sensitive plant consumers, while for OSR the presence of interfertile crops and wild relatives conducts to potential effects on rural and natural environment.

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Table 1 - *List of the identified potential effects by OMERA (column 1) versus receptor (row 1) for the oilseed rape (O) and maize (M) field trials.*

	Natural habitat	**Rural environment**	**Consumers**	**Men**	**Plant pathogens**	**Rhizosphere and symbionts**	**Soil**
Potential allergenic effects on population				Pollen (M)			
Potential allergenic effects on workers				Pollen (M)			
Potential changes to agricultural practice		Pollen (O) Seed (O)					
Potential changes to agrobiodiversity		Pollen (O) Seed (O)					
Potential changes to biodiversity	Pollen (O)						
Potential changes to edaphic fauna biodiversity							Pollen (O) Seed (O) Residues (O)(M)
Potential changes to GMP development						Plant (O)(M)	
Potential changes to GMP productivity						Plant (O)(M)	
Potential changes to rhizosphere abiotic component						Plant (O)(M)	
Potential changes to soil fertility							Pollen (O) Seed (O) Residues (O)(M)
Potential changes to soil microbe and fungal biodiversity							Pollen (O) Seed (O) Residues (O)(M)
Potential changes to structure of microbial and fungal rhizosphere populations						Plant (O)(M)	
Potential changes to structure of non-symbiotic populations						Plant (O)(M)	
Potential changes to structure of rhizosphere populations						Plant (O)(M)	
Potential changes to structure of symbiotic populations						Plant (O)(M)	
Potential changes to target pathogen host range					Plant (M)		
Potential colonisation of natural habitats	Pollen (O)						
Potential development of resistant target pathogen populations					Plant (M)		
Potential effects on biodiversity			Pollen (M)				
Potential food chain contamination		Pollen (O) Seed (O)					
Potential increase of weeds		Seed (O)					
Potential pollution of natural genetic resources	Pollen (O)						
Potential uncontrolled GMP presence in the environment	Pollen (O) Seed (O)						
Toxicity potential for consumers of new substances in pollen			Pollen (M)				

It is important to note that potential effects are not risks; indeed risk evaluation has been performed by the new FDSS. Table 2 shows the results obtained applying the new tool to the same case studies and all risks and their scores are listed.

Regarding the soil component, the oilseed rape and maize case studies show essentially the same results, obtained however by different types of responses.

Table 2 - *Identified risks by FDSS for OSR and Maize field trials. Where in the same line different kind of Risk are listed, it means that they derive directly (D) or indirectly (I) by the same rules.*

	OSR	Maize
Risk of allergenic effects on operators	low	low
Risk of allergenic effects on human population	low	low
Risk of toxicity for pollen consumers	low	high
Risk of food&feed chain contamination (D), Risk of l changes to agricultural practices (I), Risk of changes to agrobiodiversity (I)	medium	low
Risk of colonisation of natural habitats, Risk of pollution of natural genetic resources (D), Risk of changes to biodiversity (I)	medium	low
Risk of uncontrolled GMP F1 presence in the rural environment (D), Risk of increasing weed population (D), Risk of changes to agricultural practice (I)	high	low
Risk of uncontrolled F1 GMP presence in the environment by seeds (D), Risk of colonisation of natural habitats (I), Risk of changes to biodiversity (I)	low	low
Risk of changes to seed consumers populations	low	low
Risk of changes to soil microbe and fungus biodiversity	high	high
Risk of changes in crop residues detrivorous populations	high	high
Risk of uncontrolled GMP presence in the rural environment by propagation organs (D), Risk of increase in weed population (D), Risk of changes to agricultural practice (I)	low	low
Risk of changes to propagation organs consumer populations	low	low
Risk of changes to structure of symbiotic populations (D), Risk of changes to GMP growth (I), Risk of changes to GMP productivity (I)	medium	medium
Risk of changes to target pathogen host range, Potential development of resistant-target pathogen populations	low	low
Risk of changes to consumer populations	low	medium
Risk of development of resistant target consumers	low	medium
Risk of development of new pathogens	low	low
Risk of changes to structure of rhizosphere populations (D), Risk of changes to GMP development (I), Risk of changes to rhizosphere abiotic component (I)	high	medium

Indeed, while for the maize the different dispersion routes in the eQ were activated by positive responses, for the oilseed rape several "I don't know" responses were given. In these cases the software, according to the precautionary approach, considers this lack of knowledge as a source of potential risk and opens the route that represents the worst case scenario. For example, to the question "Are there any new substances or proteins in root exudates of GMP?", in the case of maize the answer was "Yes", because the Bt toxin has been identified in the root exudates of maize MON810 (Saxena *et al.*, 2004), while, in the oilseed rape GT73 case the answer was "I don't know", since no studies, analyzing the presence of new products in root exudates, have been reported,. The highlighted potential effects, changes in microbial, fungal and soil fauna biodiversity, and changes in soil fertility, are the same both for maize and oilseed rape.

In the case of insect resistant maize MON810, different effects have been reported on non target soil organisms, but only under laboratory and microcosm conditions (Zwahlen *et al.*, 2003; Brusetti *et al.*, 2004; Castaldini *et al.*, 2005; Kramarz *et al.*, 2009), while in other studies no effect has been reported (Saxena and Stotzky,

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2001a, 2001b; Griffiths *et al.*, 2006; Vercesi *et al.*, 2006; De Vaufleury *et al.*, 2007; Verbruggen *et al.*, 2012). For herbicide-tolerant oilseed rape GT73, no specific studies related to the impacts on the soil have been still published. The effects on symbiotic populations and on the variations in the root exudates, due to on changes in the bromatological composition of plant residues, are only hypothetical, because of the lack or shortage of information. The occurrence of these effects should be confirmed with specific monitoring activities, such as the analysis of abundance and diversity of bacteria and arbuscular mycorrhizal fungi, in order to obtain information on the health and fertility of soil and on the relationships established between the symbionts and the crops, also taking in account the agronomic practices adopted.
Indeed, according to the Life project program, specific monitoring activities have been conducted, during the 2010 and 2011, seasons on soil organisms.

Discussion and conclusions

The environmental risk assessment of genetically modified crops shows whether any damage to the environment can derive from the deliberate release of a genetically modified plant. It generally requires information on: characteristics and biology of the unmodified species, inserted trait and obtained phenotype, characteristics of the receiving environment, intended use, interaction between these factors (EFSA GMO Panel, 2010). The ERA, in the legislation framework in this matter, establishes the context for the identification of:
- potential hazards that may be associated with the transgenic plant;
aspects of the receiving environment that may be affected by the identified potential hazards;
- attributes of the receiving environment that may need protection;
- routes through which the identified potential hazards may come out;
- information required to assess the probability that the hazards exert an effect and that the damage occurs, and to assess the extent of that damage;
- targeted risk management strategies, based on the results of the risk characterization.

The methodologies, OMERA and FDSS, were confirmed as useful tools to carry out the various phases of the ERA, although they are still to be considered a work in progress. Theirs application, beside providing a list of potential effects and related receptors, allow the visualization of the paths, starting from the source and passing through the diffusion factors and the dispersal routes, up to potential receptors and finally allow an estimation of the identified risks. They also help to identify the missing data and information needed to assess the likelihood of the identified effects.
Comparing the results obtained with the FDSS with the previous analysis conducted with OMERA it is possible to conclude that these are coherent when considering the activated path and the identified potential effects.
The results obtained on soil highlight that rules and criteria are necessary for the implementation of field trials and that the irregular composition and the biological

fertility and quality of the soils have to be considered in a risk analysis on the release of GM plants.

In addition, the step by step analysis of the soil has provided the collection of relevant and basic information on the quality of the soils in the selected SCIs with regard to the soil structure and microbial diversity that have not yet been investigated, allowing us to assess soil quality and fertility and soil microbial activities, structure and diversity too. Such information may be useful for future monitoring programs and researches on soil status related to the agricultural activities. Farming practices, type of cultivar, tillage, soil and climatic variables are the main factors that contribute to the changes in the structure and diversity of soil microbial community. The genetic changes may have an indirect effect, through the production of root exudates, on the composition of microbial species in soil.

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