



Spatial exposure-hazard and landscape models for assessing the impact of GM crops on non-target organisms



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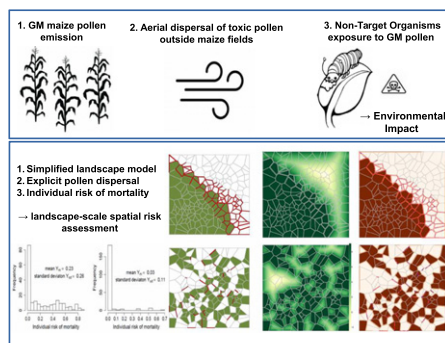
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HIGHLIGHTS

- GM crops may impact non-target organisms in agricultural landscapes.
- Spatial determinants of GM risk areas assessed using generic spatial exposure-hazard and landscape models.
- A Global Sensitivity Analysis is performed for spatial worst-case scenarii.
- It confirms the importance of space and GM pollen emission.
- It shows that the optimal spatial distribution of GM depends on our knowledge of NTO habitats.

GRAPHICAL ABSTRACT



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ABSTRACT

The cultivation of Genetically Modified (GM) crops may have substantial impacts on populations of non-target organisms (NTOs) in agroecosystems. These impacts should be assessed at larger spatial scales than the cultivated field, and, as landscape-scale experiments are difficult, if not impossible, modelling approaches are needed to address landscape risk management.

We present an original stochastic and spatially explicit modelling framework for assessing the risk at the landscape level. We use techniques from spatial statistics for simulating simplified landscapes made up of (aggregated or non-aggregated) GM fields, neutral fields and NTO's habitat areas. The dispersal of toxic pollen grains is obtained by convolving the emission of GM plants and validated dispersal kernel functions while the locations of exposed individuals are drawn from a point process. By taking into account the adherence of the ambient pollen on plants, the loss of pollen due to climatic events, and, an experimentally-validated mortality-dose function we predict risk maps and provide a distribution giving how the risk varies within exposed individuals in the landscape.

Then, we consider the impact of the Bt maize on *Inachis io* in worst-case scenarii where exposed individuals are located in the vicinity of GM fields and pollen shedding overlaps with larval emergence. We perform a Global Sensitivity Analysis (GSA) to explore numerically how our input parameters influence the risk. Our results confirm the important effects of pollen emission and loss. Most interestingly they highlight that the optimal spatial distribution of GM fields that mitigates the risk depends on our knowledge of the habitats of NTOs, and finally, moderate the influence of the dispersal kernel function.

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1. Introduction

Nowadays, one of the most successful bio-pesticides for insect control is the bacterium *Bacillus thuringiensis* (Bt) which, upon

sporulation, produces insecticidal proteins that belong to the Cry and Cyt families and are mostly active against larval stages of different insect orders (Bravo et al., 2011). This biocontrol agent or its insecticidal products can be sprayed and used in conventional and organic crop systems and, Cry expressing genes from the bacterium have been introduced into transgenic commercial crops to create genetically modified (GM) insect tolerant varieties (Mendelsohn et al., 2003). Among them, GM maize varieties producing Bt Cry proteins (e.g. Monsanto's MON810, Syngenta's Bt11 and Pioneer's 1507 Bt maize (Romeis and Meissle, 2011)) introduced for controlling the European Corn Borer, a pest that causes substantial losses, have been some of the most examined GM crops. Each of these Bt maize produces a specific insecticidal Cry protein (i.e. Cry1Ab for MON810 and Bt11 and Cry1F for 1507) whose impact on insect may depend on both the protein and the targeted species (Bravo et al., 2011). Moreover, whereas all these GM plants produce the toxic proteins in most of their plant tissues the concentrations can vary significantly between tissues within a plant, and also, between varieties (Mendelsohn et al., 2003).

Although the Bt maize primarily targets pests that are detrimental to the crop, the Bt toxin is also active against some non-target beneficial, neutral or patrimonial species that should be protected. As the Bt toxin is also expressed in pollen (Felke et al., 2010), which is dispersed by wind outside maize fields (Angevin et al., 2008) and can reach habitats of non-target organisms (NTOs) that may be exposed to the xenobiotics (EFSA, 2015; Hofmann et al., 2014), the cultivation of GM maize represents a risk toward non-target populations living in agroecosystems. While the controversial example of the impact of Bt maize on the monarch butterfly (*Danaus plexippus*) has led to several studies (Losey et al., 1999; Pleasants et al., 2001; Sears et al., 2001), the conservation of NTOs exposed to GM crops is still a debated subject (Holst et al., 2013; Kruse-Plass et al., 2017; Lang and Otto, 2010; Perry et al., 2013, 2017, 2012, 2010).

Risk assessment, which is the determination of quantitative or qualitative estimate of risk related to a recognised hazard, is a key component of public policy making for the authorization of new biological or chemical compounds used in human activities (e.g. industrial chemicals, pesticides or biotechnology-based plants) (Suter II, 2016). Regarding environmental or ecological risk assessment (ERA) several components should be considered, i) the identification and characterization of the hazard, ii) the locations of the xenobiotics sources, iii) the dispersal mode, iv) the exposed populations and v) the mode of exposure (Andow and Zwahlen, 2006). Albeit the risk assessment process requires expert advice, the use of statistical tools and mathematical models is generally fundamental for quantifying risk and testing management strategies. As xenobiotics emitted by anthropic activities generally have the potential to spread over mid or long distances, it is now recognised that efficient ERAs should be carried out at the landscape scale (Angevin et al., 2008; Focks, 2014; Gilligan et al., 2007; Graham et al., 1991; Topping et al., 2015).

Several models have been developed for assessing the risk of Bt maize on non-target *Lepidoptera* (Holst et al., 2013; Lang et al., 2015; Perry et al., 2010; Sears et al., 2001) and their outputs have been often considered for scientific advice, for instance by the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA) (EFSA, 2010, 2012, 2015, 2016). Nevertheless, none of them considered a spatial modelling framework, such as those developed for coexistence studies with explicit models for the dispersal process and the spatial structure of the landscape (Klein et al., 2003; Lavigne et al., 2008). The importance of considering explicitly space to understand and predict the behaviour of ecological systems driven by dispersal mechanisms and spatial interactions has been demonstrated by numerous works (Bolker et al., 2000; Durrett and Levin, 1994; Filipe and Maule, 2004) and spatial models are thus recognised to be central for improving ERA at the landscape level (Focks, 2014; Graham et al., 1991; Papaix et al., 2014b; Topping et al., 2016).

Therefore, the management of GM crops at the landscape level for protecting NTOs is still at an early stage of development and the improvement of models is still needed to support it (Lang et al., 2015).

In this study we concentrate on the spatial determinants of the risk for worst-case scenarios where exposed individuals are located in the vicinity of GM fields and when pollen shedding and larval emergence overlap. We first present an original spatial and stochastic modelling framework for assessing the risk of GM crops on spatially distributed NTOs in agricultural landscapes. The framework combines i) tools of spatial statistics and stochastic geometry for structuring simplified agricultural landscapes and simulating the locations of exposed individuals, ii) a method based on a convolution product and dispersal kernels for predicting the spread of toxic Bt pollen grains in the landscape, and iii) a dose-mortality relationship for assessing the risk of mortality. Then, we consider the impact of the Bt maize MON810 on the peacock butterfly *Inachis io*, a typical European NTO. We perform a Global Sensitivity Analysis (GSA) to assess numerically the influence of the spatial structure of the landscape, pollen emission, dispersal, adherence and loss on the mean and the standard deviation of the individual risk of mortality. We finish the paper by discussing our work and its interest for ERA and the management of GM crops at the landscape level.

2. Material and methods

2.1. Typical biological system

Following previous studies (Holst et al., 2013; Lang et al., 2015; Perry et al., 2012, 2010) we considered the example of the impact of the GM maize (*Zea mays*) MON810, which expresses the Cry1Ab insecticidal protein, on the peacock butterfly (*Inachis io*) for which an empirical dose-mortality relationship was established (Felke et al., 2010). *I. io* is an important European patrimonial colourful butterfly which feeds on a wide variety of flowering plants (nectariferous plants) and lay eggs on the leaves of the host plant *Urtica dioica* (i.e. nettle) (Pullin, 1986). In intensive agricultural systems nettle is essentially distributed on non-cultivated field margins. Given that the pollen of GM maize spread beyond cultivated fields and reach the habitat of patrimonial butterfly (e.g. field margins), *I. io* larva feeding on nettle can ingest toxic pollen grains and exhibit lethal or sublethal physiological damages.

2.2. Spatial exposure-hazard and landscape models

2.2.1. Overall presentation

The modelling framework consists of four stochastic and deterministic steps that are illustrated in Fig. 1 and detailed below. First, a landscape made up of GM fields, neutral fields and larvae habitat areas, where host plants are located, is drawn from a stochastic spatial process (Fig. 1A). Second, the spatial distribution of the amount of pollen, after pollen shedding and aerial dispersal, is obtained through a deterministic process that aggregates the temporal dynamic of pollen shedding in one step and integrates the contribution of all emitting sources (i.e. GM maize fields) in the landscape (Fig. 1B). Third, a map of the risk is calculated using a dose-mortality relationship (Fig. 1C). Fourth, the spatial distribution of exposed larvae is drawn from a stochastic *spatial point process* on habitat areas (Fig. 1C). Finally, the mean and the standard deviation of the risk are both extracted from the resulting distribution describing the probability of mortality (i.e. the risk) of exposed individuals in the landscape (Fig. 1D).

2.2.2. Simulation of landscapes

A stochastic landscape simulator was developed assuming that i) the landscape is made of convex plots, ii) fields can be either

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