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COLLABORATIVE PROJECT

AMIGA
Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems

D7.3 Prototypes of spatially-explicit exposure/hazard models on two case studies

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Dissemination Level		
PU	Public	**X**
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Summary

The present deliverable is part of Task 7.3 "**Design of exposure and hazard models to assess the interest of predictive models to drive PMEM by identifying hotspots situations". In order to** assess to what extent exposure-hazard models may help risk managers set up efficient monitoring schemes, this task considers two specific crop-trait-pest situations:

- Design of a spatially-explicit exposure model to assess the impact of *Bt* maize on the mortality of non-target Lepidoptera;
- Effect of cropping systems and of herbicide management regimes on weed abundance and diversity.

In the case of non-target Lepidoptera, the concept of the exposure model developed by Perry *et al.* (2010, 2013) has been expanded in order to (i) design a spatially-explicit exposure model for both the pollen dispersal and the spatial dynamics of Lepidoptera and (ii) account for the temporal dimension of the exposure model (phenology of non-target Lepidoptera and different flowering periods of maize. To date, the specifications of the components of such a model have been defined and are described in this deliverable. A sensitivity analysis has been carried out to identify those landscape factors that may increase or decrease the mortality at the landscape level.

As for the herbicide tolerant case study, the existing generic FlorSys model (Colbach, 2014) has been extended to (i) adapt it to maize-based cropping systems and their specific weed flora, (ii) to consider a multi-field landscape situation and (iii) to be able to use FlorSys to assess weed resistance evolution. The adaptation of Florsys to AMIGA needs is described in "D7.4 Adaptation of FlorSys to maize cropping systems" and is not reported here.

Design of a spatially-explicit exposure-hazard model for Bt maize and some non-target Lepidopteran.

This short report describes the general framework implemented within AMIGA to design generic spatially-explicit exposure models and its implementation status in April 2015. As this subtask started later than expected, it is still ongoing and an updated version of the model will be included in the deliverable D7.5 "Report on the potential use of exposure-hazard models to optimize monitoring sampling schemes ».

The task aims to design, build and implement spatially-explicit exposure models in order to assess the impacts of Bt maize introduction on Lepidopteran at a regional scale.
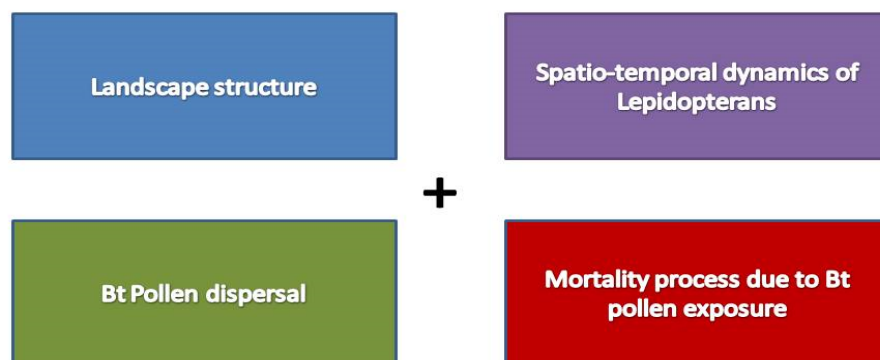
Main

Figure 1: Main components for assessing the impacts of Bt maize cultivation on non-target organisms at a regional scale

components of the system

In order to predict and analyze the impacts of Bt maize fields on non-target organisms within a landscape, four *a priori* main components were identified (Fig. 1):
- the structure of the agricultural landscape;
- the spatio-temporal dynamics of non-target lepidopterans;
- the dispersal of Bt-maize pollen within the landscape;
- the toxicological processes involved during exposure and their consequences on the physiology of exposed organisms (e.g. mortality, decrease in the fitness of the population).

These four major components may be seen as four interacting modules, which can be developed independently from each other to provide useful tools for model-based risk assessment, and to contribute to the understanding of fundamental and applied ecological questions.

Risk assessment and time scales in the context of GM crops

Bt maize might affect non-target organisms in various ways and the consequences of these potential impacts may be detected at different time scales. For instance, the exposure of non-target organisms to the pollen emitted by GM plants expressing the insecticidal Cry1 protein may kill these organisms or/and affect their fitness and dispersal capacities. While the impact of Bt maize through death of non-target individuals can be assessed on an annual basis, the sublethal effects of GM crops on non-target population through the alteration of the fitness and dispersal capacities would require long-term (i.e. pluri-annual) assessment.

Based on the preliminary studies carried out by Perry et al. (Perry, 2010, 2012), we first focused on the development of modules to assess the short-time effects of Bt maize on Lepids. However, knowledge on ecological, biological and agronomic processes involved in the direct mortality of larvae as well as the codes developed here would provide a strong basis to design models to conduct a long-term assessment of the risk of Bt maize cultivation on non-target populations.

Spatial structure of the agricultural landscape

As it will impose strict conditions on the spatio-temporal dynamics of non-target populations, and, on the spatio-temporal distribution of maize pollen present on their living area, the structure of the agricultural landscape is a major component that needs to be taken into account to assess the impact of Bt crops at a regional scale. Using tools from spatial statistics and stochastic geometry, we developed two modules to simulate stochastic landscapes.

The first one generates landscapes where the user can control:
- the number of maize fields (GM+ non GM) within the landscape (*marked point process*);
- the proportion of GM crops among maize fields;
- the spatial aggregation of GM fields (G*aussian process with spatial autocorrelation and thresholding*);
- the probability of a field margin of having host plants where Lepids can lay there eggs (*binomial distribution*);
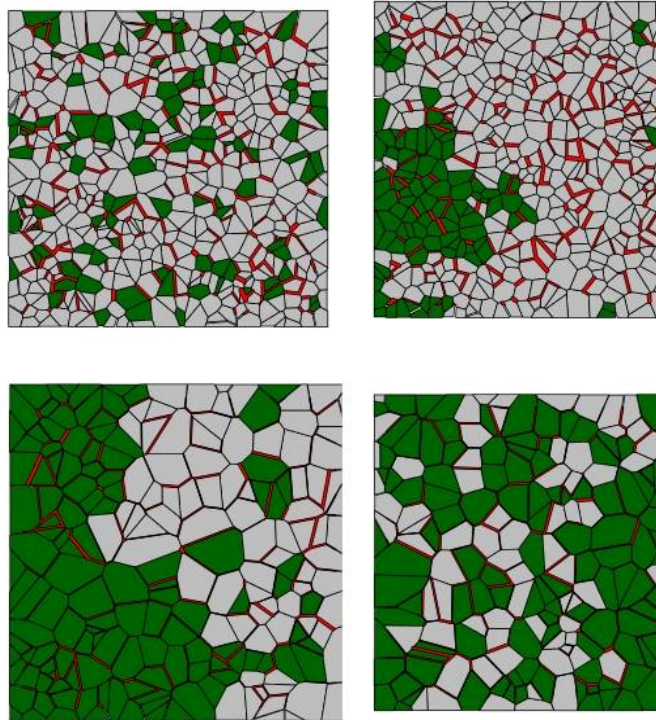- the width of these margins with host plants (*Gamma distribution*).

Figure 2: Theoretical landscapes obtained from our stochastic landscape generator. Green polygons represents fields with Bt maize whereas grey ones correspond to non-Bt fields. Margins with host plants are represented in red.

The first version of this landscape generator uses Voronoi tessellations to create the geometry of fields within the landscape (Fig. 2). However, other tessellations such as rectangular or T-tessellation may be used instead.

The second module, fully independent of the above one, simulates the spatial distributions of host and nectariferous plants on the edges of fields, allowing the user to control the minimum distance between « host margins » and « nectariferous margins ».

Pollen dispersal within landscapes

Several mechanistic and statistical methods have already been developed and applied to predict the dispersal of pollen. Each of these approaches has its own strengths and weaknesses. The use of dispersal kernel functions, appears to be the most appropriate method to predict pollen dispersal for the AMIGA project. A dispersal kernel is a probability function describing the spatial distribution of spatial units originating from a common source. Dispersal density kernels can be either empirical functions that can predict quite well observed patterns but with poor mechanistic interpretations, or, theoretical-mechanistic functions that often failed to adequately predict observations. Dispersal kernels allow to predict the spatial distribution of propagules dispersed from one source. Within landscapes, where many pollen sources are present, the spatial distribution of propagules corresponds to the sum of all individual sources and can be predicted by using a convolution product:

$$R(x,y,t) = \iint_\Omega E(x',y',t)K(|x-x'|,|y-y'|)dx'dy' = K \otimes E(x,y)$$

Where Ω is the region over which the pollen is dispersing, E(x,y) is the function that describes pollen emission at point x and time t and K(x,y) is the dispersal kernel (empirical or theoretical) that describes the movement of particles from x to y.

The choice of a suitable kernel function is crucial to predict the spatial distribution of pollen, given a landscape with maize, and different functions (e.g. heavy-tailed versus light-tailed kernels) could lead to significantly different patterns. For the project we first concentrated on the use of the quasi-mechanistic Normal Inverse Gaussian (NIG) kernel, that has already been fitted with field data (Klein et al., 2003). The NIG kernel corresponds to a light-tailed-exponential kernel, which disperses propagules mostly on short distances away from sources, but it exhibits an anisotropic heavy-tailed along the wind direction when its intensity is sufficient for transporting pollen over long distances. The NIG kernel is expressed as:

$$NIG(\lambda_z, \lambda_x, \lambda_y, \sigma_x, \sigma_y, x, y) = \frac{\sigma_x \sigma_y e^{\lambda_z}}{2\pi} \frac{(q(x,y)^{-0.5} + p^{0.5})}{q(x,y)} e^{-\sqrt{pq(x,y)}} e^{\sigma_x \lambda_x x + \sigma_y \lambda_y y}$$

$$\text{with } p = \lambda_z^2 + \lambda_x^2 + \lambda_y^2 \text{ and } q(x,y) = 1 + \sigma_x^2 x^2 + \sigma_y^2 y^2$$

Where (x,y) is the pollen deposition in Cartesian coordinates relative to the emission position and $\lambda_x, \lambda_y, \lambda_z, \delta_x, \delta_y$ are parameters adjusted by wind conditions as follows:

$$\lambda_z = 0.027 \frac{h}{0.831} \ ; \ \lambda_x = 0.165 \frac{h}{0.831} \frac{\mu.\cos(\theta)}{2} \ ;$$

$$\lambda_y = 0.165 \frac{h}{0.831} \frac{\mu.\sin(\theta)}{2} \ ; \ \delta_x = \delta_y = 0.499 \frac{0.831}{h}$$

With h the difference in height between GM male flowers and host plant where butterfly will lay their eggs, and (μ,θ) are the daily average wind speed and direction at ten meters high for each day.

While the NIG kernel have already been validated against data and seems to represent the dispersal of maize pollen reasonably, other functions (e.g. empirical heavy-tailed kernels) could be used and easily implemented to assess the impact of differing hypothesis (e.g. significant long distance dispersal) the spatial distribution of Bt pollen within agricultural landscapes. Following the recent publication of an extensive set of pollen dispersal data by Hofmann et al. (2014), we have also used the Power pollen dispersal curve, as estimated in their paper. Although, this curve integrates to infinite, it provides a good estimation of the amount of pollen dispersed over distances up to 4.4 kilometers (See also EFSA, 2015).

Spatio-temporal dynamics of non-target Lepidopterans

Two main approaches can be distinguished for describing the dynamics of non-target organisms: the individual-based Lagrangian approach that describes the dynamics of each individual among the population and the population-based Eulerian approach (Dupont et al.,

2006). Both methods can have advantages and disadvantages and require the use of different mathematical techniques. One of the strengths of computational intensive individual-based models is their capacity to predict population dynamics better than population models that are designed to predict the evolution of large populations. For modeling the dynamics of non-target butterflies in agricultural landscapes, the asymptotic (N⟶∞) assumption often inherent in population-based models is likely to fail, especially for those endangered species that express low abundance.

Lepidopterans usually reproduce sexually and are oviparous (egg-laying on host plants). After several instars, the larvae (or caterpillars) turn into pupa (or chrysalis) from where the imago (adult) emerge. In this work we considered only females that produce eggs (and thus indirectly larvae) and we split the life-cycle into two stages or states: adults (dispersal stage) and larvae (quasi-immobile stage susceptible to Bt pollen exposure). The spatio-temporal dynamics of non-target organisms was described using the individual-based model below:

- the movement of adults (adult-moving-state) is described by a system of Stochastic Differential Equations (SDE):

$$\begin{pmatrix} dX(t) \\ dY(t) \end{pmatrix} = \mu(x, y, t)\, dt + \sigma(x, y, t) \begin{pmatrix} dW_x(t) \\ dW_y(t) \end{pmatrix}$$

Where dX and dY are the incremental step sizes in the x and y directions, μ and σ are respectively the drift and diffusion parameters, W_x and W_y are independent Wiener processes describing Brownian motions. One advantage of this mathematical description of individual movement is that the equivalent population model, derived from partial differential equations (PDE), can be directly obtained by using the Fokker-Planck equation.

The movement of Lepidopterans can be influenced by the structure of the landscape. For instance, while adults may be attracted by geographical areas with nectariferous plants and subsequently by zones with host plants (attractors), they could be repulsed or blocked by hedges. In order to take into account the influence of attractive and repulsive elements of the landscape, we assumed that individuals are under the influence of a potential field:

$$\mu = -\nabla H$$

where H is a suitable potential for each attractor or repulsor in the landscape.

- the reproduction is described as a branching process and the number of immobile-eggs-larvae produced by each female is drawn from a Gamma distribution;

- the death of individuals is governed by a Poisson process: individuals (adults and larvae) have a natural mortality (rate λ) and larvae can also die after Bt pollen exposure (rate γ) if pollen is spread locally.

This modelling framework that describes the spatio-temporal dynamics of non-target Lepidopterans within an agricultural landscape is quite generic and quite flexible. As a consequence it will be straightforward to implement, for instance, multi-voltinism or the effect of other stressors on individuals (additional mortality).

Toxicological processes involved during exposure and their consequences on the physiology of exposed organisms

The consumption of pollen grains containing the Cry1 protein might have various effects on individuals and their progeny. So far, scientists have mostly investigated the mortality of larvae that are directly exposed to Bt pollen on their host plants. Available experimental data have been analyzed through regression that gives a simple concentration-dose relationship based on a logit model:

$$\text{logit}(p) = \alpha + \beta \log_{10}(d)$$

Where p is the proportion of individuals that suffers mortality, d is the concentration of Bt-Maize pollen expressed in pollen grains cm^{-2}, α and β are parameters estimated from experimental bio-assays.

These mortality-dose relationships are based on strong hypotheses about the toxicological processes involved and the physiology of exposed individuals that are likely to be violated : e.g., variability among individuals, cumulative effects not accounted for, toxicodynamics within organisms, sublethal effects that may affect survival of populations over subsequent years. Within AMIGA, we have used the concept of ecotoxicological modelling as presented by Aschauer et al. (2007) to account for the cumulative effect of exposure to Bt pollen. This is done by using the concept of internal concentration, which aims at representing the dynamics of pollen ingestion, not just the total amount of pollen to which larvae have been exposed. Also, the differential temporal dynamics of pollen emission across maize fields is considered by taking advantage of gene flow modelling studies carried out in the context of coexistence (Angevin et al., 2008).

These models will allow us to test various hypotheses and assess the importance of refining toxicological processes at individual scale to predict the impacts of Bt maize at a population scale.

Additional reports and publications

Based on the first two components (landscape patterns, pollen dispersal, a first sensitivity analysis has carried out without considering the temporal dynamics of the pollen shed and of larvae development. This first step aimed at ranking the effects of various factors affecting the mortality of Lepidopteran at the landscape level: pollen production, allocation of GM fields across landscape, size and management of field margins, etc. The results of such a sensitivity analysis are reported in a scientific paper to be submitted during the Summer 2015 (Leclerc M., Soubeyrand S., Walker E., Roques L., Messéan A. A spatially explicit modelling framework for assessing the risk of Bt maize on non-target Lepidoptera at the landscape scale, in preparation).

A generic software package, encompassing the four components above, is being developed and should be easily implemented for a wide range of butterfly populations.

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