



Project Number 289706

COLLABORATIVE PROJECT

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

Start date of the project: 01/12/2011

Duration: 54 months

**D7.5 Report on the potential use of exposure-hazard models to
optimize monitoring sampling schemes**

Organisation name of lead contractor for this deliverable: **INRA, Institut National de la Recherche Agronomique, France**

Project leader: Dr. Salvatore ARPAIA, ENEA - Centro Ricerche Trisaia Rotondella (MT), Italy

E-mail: Salvatore.arpaia@enea.it

Project website address: www.amigaproject.eu

Project funded by the European Commission within the Seventh Framework Programme (2007-2013)		
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)	

Introduction

Due to the high diversity of receiving environments, as well as of management and production systems across Europe, the Environmental Risk Assessment (ERA) of GM crops should consider a range of representative scenarios but the ERA cannot cover all possible situations. In addition, unanticipated long-term effects may occur, either because of a delayed response of some effects, or as the result of an inevitable increase in spatial and temporal complexity for large-scale cultivation. Also, receiving environments and management systems are continuously evolving over time. In this context, the Post Market Environmental Monitoring (PMEM) is of major importance :

- (i) to confirm the assumptions 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 initial ERA”,
- (iii) to detect any unexpected adverse effects.

Monitoring potential environmental GM crop effects therefore requires a complex approach : (i) different spatial and temporal scales need to be considered, (ii) different land use and cultivation systems are concerned, (iii) potential long-term GMO effects (e.g., on farmland biodiversity) have to be investigated and (iv) unforeseen effects may occur as the frequency and scale of GM crop cultivation changes and/or increases.

While the EFSA ERA and PMEM Guidance Documents (EFSA, 2010a ; EFSA, 2011) were updated to take stock of recent advances in scientific research and of experience gained from the first generation of GM crops grown worldwide, little experience had been obtained on PMEM of commercial releases and there is still a need to develop a coherent framework, validated methods and tools to better integrate ERA and PMEM and help implement cost-efficient monitoring schemes.

In this context, a major progress is expected from modelling. Indeed, exposure models can be used to support the assessment of potential effects of GM plants on agroecosystems and to drive monitoring schemes as they make it possible (i) to explicitly account for the variability of factors driving environmental impacts and (ii) to consider upscaling effects in time and space. They can also assess the possible impacts of those uncertainties that inherently exist for several parameters and/or endpoints. Thus, modelling approaches could be of high value to identify « hotspot » situations, i.e., identify in which receiving environments and under which management systems possible adverse impacts are likely to occur and/or are maximal. Subsequently, monitoring schemes could primarily target those specific situations where potential adverse effects are most likely to occur. So far, existing exposure models are mostly local and static (e.g., Perry, 2012, Holst 2013 for impacts of Bt maize on non-target Lepidoptera) while upscaling effects require considering the spatial and temporal dynamics of management systems (landscape patterns, GM uptake, cropping systems, etc).

AMIGA have been developing spatially-explicit exposure models to assess their feasibility to support risk managers to make decisions on management scenarios as well as to set up optimal Post-Market Environment Monitoring schemes. Two case studies have been considered:

- Predict the effect of cropping systems and of GM-based herbicide management regimes on weed abundance and diversity as well as the resulting impacts on biodiversity at large;
- Assess the impact of *Bt* maize on the mortality of non-target Lepidoptera at the regional level.

Case study on the effect of cropping systems and of herbicide management regimes

Weeds are harmful for crop production but are also an important component of vegetal biodiversity in agricultural landscapes and contribute to feeding other components of agricultural biodiversity. In parallel, there is a trend to reduce herbicide use and their impacts on the environment. Overall, one must reconcile agricultural production, biodiversity in arable systems and reduction of environmental impacts linked to herbicide use. In this context, the potential role of herbicide-tolerant crops, be them genetically modified or not, is under debate. They can improve weed control efficiency while minimizing environmental impacts (Pidgeon, 2003) but they also induce changes in practices (crop rotation, soil tillage) that, in turn, might have detrimental effects (Lamichhane, 2016). In addition, they could also increase the selection pressure on weed communities that can consequently evolve resistance (Neve, XXX). These are long-term

and context-dependent effects that can only be assessed by considering the “cropping systems” dimension over time.

To assess the indirect and long-term effects of introducing GM crops in maize-based cropping systems, AMIGA has used and adapted the weed life-cycle model FLORSYS. FLORSYS (Colbach, 2010) quantifies the effects of cropping systems and pedoclimate on weed dynamics as well as indicators of weed-related biodiversity (species richness and equitability, trophic resources for birds, insects and pollinators) and crop production loss (yield loss, harvest contamination, harvesting problems, field infestation).

During AMIGA, FLORSYS was adapted to account for the specific objectives of the project (see D7.4) :

- account for the specific characteristics of the maize weed flora (e.g., traits related to the competitiveness with the crop) in addition to those weed species that were previously parametrized in FLORSYS;
- include a Lepidoptera indicator in addition to other indicators of weed impact on crop production and biodiversity ;
- assess the development of weed resistance to glyphosate;
- develop a landscape version of FLORSYS, including a seed dispersal component, in order to assess the impact of landscape management scenarios on the overall farmland biodiversity at the landscape level.

FLORSYS was then used to carry out simulation studies aiming at predicting the impacts of various management scenarios that could result from the introduction of GM maize in European agriculture. These simulation studies focused on two GM maize variety types, one expressing *Bacillus thuringiensis* toxins against target pests (Bt) and the second including a glyphosate herbicide tolerance trait (HT). Two contrasting maize-growing sites were studied: Aquitaine, a region in South-Western France, and Catalonia in North-Eastern Spain. Typical regional cropping systems containing maize were identified for each site from expert knowledge and/or database recording agricultural practices. GM scenarios were based on expert knowledge, literature and current Spanish experience. Probable scenarios (involving conventional, Bt or HT maize) were simulated over 28 years for each region, and repeated with 10 different regional random weather series. An additional series of 5-7 scenarios per region was run to decorrelate factors, and make it easier to identify the cultural practices responsible for changes in weed flora, biodiversity and production. Although Bt maize does not change weed control strategies as such, it may induce indirect changes of cropping systems (e.g., rotation). Results for Bt maize are not reported here but can be found in Bürger et al. (2015).

Cropping system	Aquitaine							Catalonia						
	Weed-mediated biodiversity				Harmfulness			Weed-mediated biodiversity				Harmfulness		
	Species richness	Species equitability	Bird food	Carabid food	Crop yield loss	Years to mutant advent	Species richness	Species equitability	Bird food	Carabid food	Crop yield loss	Years to mutant advent		
1 Diverse rotation	16.41 A	0.26 HI	5.83 C	2.92 C	33.65 C	7.02 BC	18.28 D	0.40 CB	3.83 J	4.87 D	58.93 C	10.10 A		
2 Diverse with HT maize.	16.67 A	0.28 HG	5.76 C	2.80 D	28.75 D	8.45 BC	16.71 F	0.39 CB	3.24 K	4.25 F	41.89 E	9.49 BA		
3 + no plough.	9.92 G	0.44 E	3.06 F	2.11 E	13.08 F	17.38 A	14.94 G	0.42 A	6.29 E	5.52 C	77.09 A	1.50 C		
4 Wheat/HTmaize	12.66 E	0.35 F	0.95 G	2.02 E	19.01 E	10.85 BA	20.07 A	0.39 CB	4.12 I	4.53 E	64.12 B	7.40 BAC		
5 + no plough..	15.91 B	0.29 G	6.34 B	4.63 B	48.77 A	2.61 DC	19.84 A	0.40 B	6.93 D	5.65 C	57.37 C	1.00 C		
6 HT maize monoculture	12.51 E	0.16 J	5.59 D	1.82 F	1.06 G	20.63 A	16.86 F	0.38 CB	7.08 DC	2.31 I	6.43 F	8.46 BAC		
7 + early sowing	11.79 F	0.44 E	5.89 C	1.17 G	-6.18 H	9.67 BA	15.11 G	0.33 E	7.15 C	1.92 J	2.59 G	12.33 A		
8 + late sowing	14.08 D	0.34 F	5.56 D	2.13 E	0.50 G	8.71 BC	19.02 CB	0.18 F	5.83 G	2.83 G	0.30 G	6.97 BAC		
9 + no plough.	8.72 H	0.69 B	5.34 E	0.63 I	-0.05 G	11.40 BA	13.35 I	0.43 A	6.40 E	0.84 K	0.34 G	1.78 BC		
10 + no plough + early sowing	7.31 I	0.75 A	5.25 E	0.02 K	-9.13 I	> 28	13.98 H	0.34 ED	6.07 F	0.93 K	-0.05 G	8.73 BAC		
11 + no till	15.33 C	0.27 HI	9.58 A	8.68 A	39.07 B	1.00 D	18.23 D	0.38 C	9.79 A	8.81 A	49.90 D	4.00 BC		
12 + no till + 2nd glyphosate	15.14 C	0.25 I	9.51 A	8.63 A	37.31 B	1.00 D	19.21 B	0.40 CB	9.52 B	8.56 B	40.86 E	0.96 C		
13 + no till + 2nd gly. early sow.	15.16 C	0.26 I	9.56 A	8.67 A	38.05 B	1.00 D	18.71 C	0.36 D	9.71 A	8.75 A	42.72 E	2.50 BC		
14 + catch crop killed with glyph.	5.26 K	0.59 D	0.05 H	0.23 J	-0.13 G	> 28	12.53 J	0.34 E	1.32 L	1.80 J	1.12 G	9.99 BA		
15 + catch crop killed with tillage	6.09 J	0.64 C	0.09 H	0.91 H	-0.04 G	2.25 DC	17.21 E	0.16 G	5.64 H	2.49 H	0.64 G	10.05 A		

Table 1. Results of simulations concerning the indirect effects of the introduction of HT maize into existing European cropping systems. 15 different scenarios were considered. Evolution after 28 years is reported for a series of indicators related to weed-mediated biodiversity (species richness, species equitability, bird food, carabid food) and harmfulness (crop yield loss, resistance evolution).

The simulations (table 1) showed that the likely changes in practices accompanying the introduction of HT maize varieties affected weed flora as well as weed-related biodiversity and crop production loss, but that the consequences depended on the regional specificities. Most of these consequences were caused by the simplification in the cropping systems made possible by HT maize, rather than the associated use of a

non-selective herbicide (i.e., glyphosate). Simplified tillage or no-till increased weed harmfulness. Conversely, no-till cancelled part or all of the nefarious effects on biodiversity of simplified rotations (maize/wheat and maize monoculture), particularly by improving trophic resources offered by weeds to birds, insects and pollinators. Overall, biodiversity was less affected by simplified rotations in Catalonia where the initial weed flora was richer and more equitable. Delayed maize sowing reduced weed harmfulness and biodiversity, except food offer for insects and pollinators whose pertinent feeding period covered spring and summer (vs. winter for birds).

As for the potential of weeds to evolve resistance, the simulations showed that, if the reliance on a single herbicide increases selection pressure, the other components of cropping systems (soil tillage, rotation of crops, crop management) have a higher effect than the herbicide cocktail itself and might even prevent/delay the advent of herbicide resistance.

It should be highlighted here that the above effects were observed by considering only the intended (tolerance to glyphosate) as well as the expected unintended effects (change in soil tillage and crop rotation) of HT crops ; possible unintended effects on the characteristics of the GM plant (germination capacity, fitness) were not accounted for. However, FLORSYS explicitly includes these characteristics in its life-cycle model and the implications of any consistent changes in such traits could easily be assessed. It is likely that the magnitude of the effects of differences between the GM plant and its conventional counterpart will depend on the specific receiving environment considered.

Overall, the simulations studies demonstrated that :

- the change in herbicide regimes is not the only component of cropping systems to consider when risk managers want to assess the possible impacts of GMHT crops on farmland biodiversity ;
- similar conclusions can be drawn for weed resistance that was only partly driven by the novel herbicide and was dependent on other components of cropping systems (e.g. no till).
- the effects also depended on the initial characteristics of the receiving environment in which the GM crop is to be grown : initial weed diversity, soil, climate, management systems.

The implications for ERA/PMEM are the following ones :

- Given the diversity of receiving environments across Europe, the actual impacts of HT crops can only be assessed on a case-by-case basis ; the scenario analysis as recommended by the EFSA ERA Guidance Document (EFSA, 2010) can help frame the possible range of impacts but no conclusion that would be valid for all receiving environments can be drawn ; nevertheless, risk assessors can identify those drivers and characteristics of cropping systems that should be considered by risk managers when considering the introduction of HT crops in their countries/regions ; risk managers should assess whether the impacts will be lower, equal or higher than those identified in the initial pre-market assessment ;
- Predictive models such as FLORSYS can help risk managers predict impacts at the local level but its use on a routine basis would require the development of a simplified model (a « meta-model ») that would make it feasible to be used by risk managers and practitioners ; such a decision-support tool is currently under development at INRA ;
- As for PMEM, the main implication is that PMEM should be targeted and that, when deciding which biodiversity component to monitor after introducing GM crops to detect any undesirable effects, the choice will vary with the region ; in our simulation study, in Aquitaine, the bird-food indicator appeared to be the most affected trophic resource, and thus birds would be the component to focus on in ex-post monitoring ; conversely, in Catalonia, bird food was the least affected, and monitoring might rather focus on pollinators ; these conclusions are still tentative and dependent on the model's domain of validity ;
- Also, the simulations were carried out by assuming that the characteristics of the cropping systems and management systems would not change over a period of 28 years, this is obviously unlikely to occur and results would actually be less contrasted between scenarios; however, long-term predictive models could be first used to identify key drivers and sensitive endpoints while the same model can be used to update on a real-time basis the prediction of impacts on the environment ; a real-time monitoring of impacts based on the actual practices and update of consequences can be implemented ; should monitoring observations be done, they can also be used to validate/correct the assumptions of the model.

Our results demonstrate that risk management measures should be adapted to the specific cropping

systems into which HT maize is introduced. Similarly, our results suggest that monitoring needs to be adapted to actual management systems and our modelling approach help identify those endpoints that should primarily be monitored.

Impact of *Bt* maize on the mortality of non-target Lepidoptera at the regional level

Various *Bt*-crops have been developed to produce insect-resistant maize, expressing a toxic protein for target pests, e.g., *Bt* maize expressing the Cry1Ab toxin against the European Corn Borer (Holst et al., 2013). Although this *Bt* maize primarily targets those pests that are detrimental to the crop (European Corn Borer and/or Mediterranean Borer), the *Bt* toxin is also active against some non-target butterflies that should be protected (e.g., *Inachis io*). The *Bt* toxin is also expressed in pollen and, through wind-mediated pollen dispersal outside GM maize fields, its can reach habitats where non-target populations are used to feed. Such populations can therefore be exposed to the *Bt*-toxin (see Perry et al., 2012), leading to mortality or affected fitness.

The effects on non-target organisms are driven by numerous factors that may be used to mitigate the actual risk: density and distribution of GM maize fields, synchronicity between pollen emission and temporal dynamics of non-target populations, spatial feeding behaviour of insects, sensitivity of insect species to the toxin. As these factors highly depend on local receiving environments, modelling approaches are needed to assess the overall risk.

The overall objective was to design a modelling tool that would make it possible, for every receiving environment, (i) to assess to what extent *Bt* maize can be cultivated with no significant impact on populations of non-target species and, if any impact, (ii) to assess whether some management measures can reduce the level of risk at an acceptable level. So far, no study has combined the effect of temporal dynamics with the spatial nature of agricultural systems. We hypothesize that the spatial arrangement of maize fields and the distribution of host plants across landscape may greatly impact the level of risk and that a spatially-explicit dynamic model would help risk assessors and risk managers to make science-based decisions.

To this purpose, the concept of the exposure model developed by Perry *et al.* (2012) has been expanded in order to (i) design a spatially-explicit exposure model for both the pollen dispersal and the spatial dynamics of Lepidoptera, (ii) account for the temporal dimension of the exposure model (phenology of non-target Lepidoptera and different flowering periods of maize and (iii) better account for ecotoxicological effects.

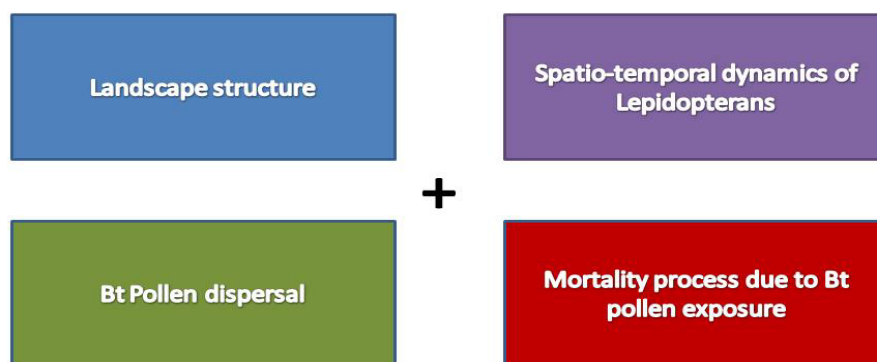


Figure 1: Main components for assessing the impacts of *Bt* maize cultivation on non-target organisms at a regional scale

A generic package has been developed (BrisKar) and includes four components (see figure 1):

- Landscape structure: actual landscape patterns (maize fields, field margins, other fields and habitats) are explicitly described ;

- Non-target Lepidoptera dynamics. The exposed larvae are described by a marked spatial point process whose parameters can be adapted to biological data;
- Pollen dispersal. The dispersal of pollen is modelled as a convolution between the sources of pollen (maize fields) and a dispersal kernel; actual pollen deposition on host plants results from accumulation of pollen and loss processes: accumulation results from successive dispersal/loss events and daily climatic conditions (rain) drive loss processes;
- Toxicokinetic/-toxicodynamic (TKTD). The temporal dynamics of the internal concentration of toxins within individuals (toxicokinetic part) is described by a model based on an ordinary differential equation; the internal concentration of toxins governs the occurrence of lethal or sublethal effects.

The use of the generic package has been illustrated by considering a simplified but typical case study. We considered the case of a non-target butterfly species whose adults lay eggs on host plants that occur in margins of agricultural fields. Eggs become caterpillars (i.e. larvae) that eat leaves of host plants (e.g., nettles). If the larval stage overlaps with the flowering of Bt maize (i.e. the period during which the Bt maize plants release pollen containing the Bt toxin), the larvae ingest the Bt-toxin through pollen grains which are dispersed from Bt maize and deposited on host plants.

To illustrate our approach, we applied it to the real and simulated landscapes as shown in Figure 2. In both cases, the landscape cover a 5000x5000m spatial domain, which was rasterized for the computation of the dispersal of contaminants into a regular squared grid with 210 x 210 pixels. The real landscape contains 184 source « polygons » (corresponding to GM maize fields), 145 receptor polygons (corresponding to meadows or crops with host plants for the exposed individuals) and 131 neutral polygons. In this landscape, field shapes are real, but the field types are arbitrary. The percentage of GM fields, namely 40%, is consistent to patterns observed in agricultural areas with moderate density of Bt maize. For the simulated landscape, the Voronoï approach was used to generate polygons/fields.

Field margins (« receptor cells ») were generated with the similar Voronoi tessellation approach. Since local movement of individual larvae on host plants are negligible in comparison to typical dimension of landscape components, we considered that susceptible individuals were all immobile and could thus be represented by a point process. For the simulations, 100 larvae (blue crosses in Figure 2) were randomly and uniformly distributed within field margins.

Emergence dates (i.e. dates of birth) were independently drawn from a uniform distribution over the simulation period (June and July). The duration of the larval stage was set to 20 days (Holst et al., 2013), and the threshold for the lethal dose of Bt-pollen was set to 15 pollen grains for all exposed individuals. At each time, pollen dispersal was simulated from the sources of pollen bearing the Bt toxin (GM maize fields). For each field, the beginning of pollen shed was randomly selected from a uniform distribution over days and the pollen shed dynamics over time was the one described by Angevin et al. (2008).

A dispersal kernel was applied to each pollen emission source and integrated on all fields to produce maps of dispersed pollen at each time. Three different dispersal kernels can be implemented in the package. Here, we used the Normal Inverse Gaussian kernel with the same parameter values as in Angevin et al. (2008). Local pollen deposited on host plants was calculated from the actual pollen dispersal at each point weighted by the adherence of pollen on leaves arbitrarily fixed at 40%. At each time, this deposited pollen accumulates with previously deposited pollen on leaves. Pollen loss due to rainfall was accounted for to calculate the everyday concentration of pollen on leaves. We chose a daily rainfall series covering the period from June 1st to July 31st of 2013.

Simulations were carried out to validate the functionalities of the package. Figures 2 and 3 illustrate typical outputs of brisKar and demonstrate the added value of brisKar over existing approaches (spatially-explicit landscapes and individual-based model approach).

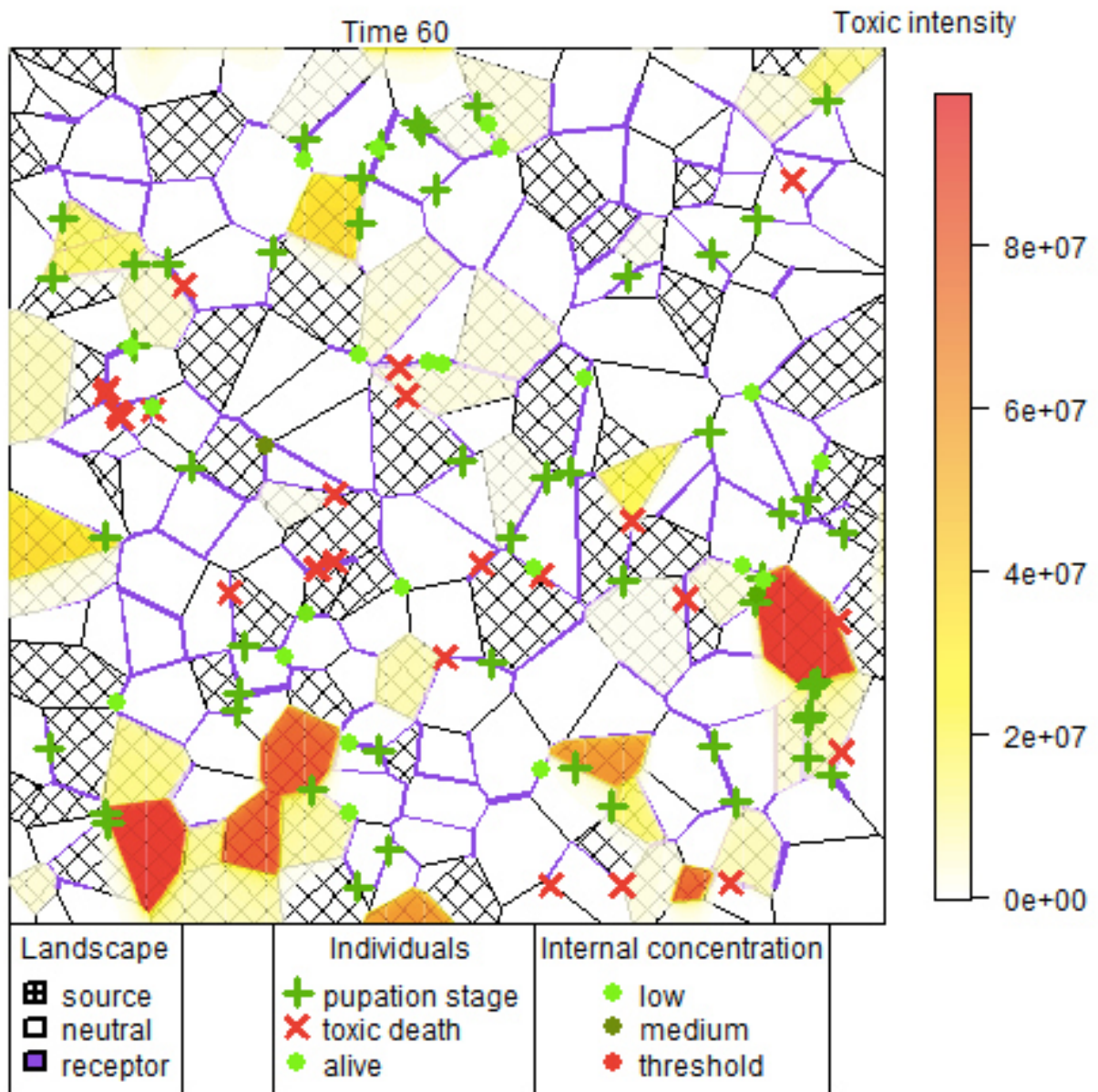


Figure 2. Local pollen (number of pollen grains) after dispersal over the landscape at time 60 of simulation. Individuals are represented by points when alive, green crosses when pupation stage reached and red crosses when death due to high toxic internal concentration. For alive individuals, the level of internal concentration of toxic is given by the color of the point.

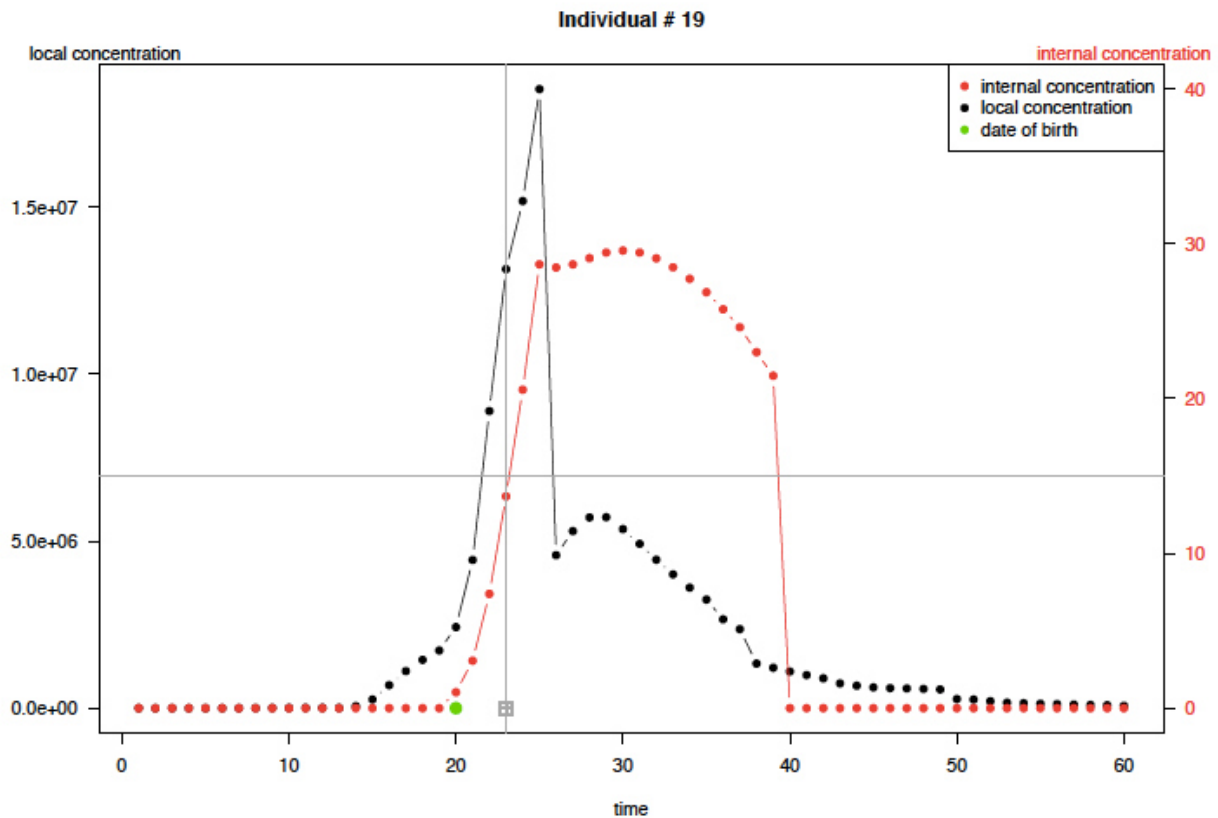


Figure 3. Example of larva toxic internal concentration with varying impact of toxic pollen (local pollen in black and individual toxic concentration in red). In this case, the lethal threshold was reached: the larva is dead because of toxic levels of Bt-toxin. The horizontal grey bar corresponds to the lethal threshold fixed at 15 grains of pollen for internal concentration, and the vertical grey bar corresponds to death time due to overtaken internal concentration.

So far, brisKar is still a prototype and the first simulation studies were carried out with generic values of parameters. BrisKar is currently used to develop a more specific and realistic case study :

- small agricultural area near Foixa in Catalonia with the actual allocation of GM and non-GM maize observed between 2004 and 2008 ;
- flowering periods of each maize field and weather data as recorded ;
- average larval stages of *Inachis io* measured in this part of Catalonia are considered ;
- actual location of larvae in field margins are still simulated (no real observation available) but clusters of larvae are considered.

This will make it possible to assess the real added value of using a spatially-explicit model against the approaches developed by Perry et al. (2012) and Holst et al. (2013). Various scenarios of spatial allocation of Bt maize fields and management of sowing dates will be assessed as for their potential to reduce exposure of non-target species larvae and, consequently, their mortality.

Similar to the FlorSys case study above, brisKar could be used to help risk managers estimate the actual risk under their local conditions and undertake appropriate management measures, such as setting up isolation distances from protected areas or recommend allocation of GM maize (e.g., clustering) that would minimise the impacts on populations at risk.

Mortality of non-target organisms at the regional level would also depend on the other characteristics of the landscape (presence of natural habitats, other crops, structure and management of field margins), hotspots can also be identified and drive the selection of those sites where the butterfly monitoring protocol as developed in AMIGA could be used. Similarly to FlorSys, a simplified version of the model should be envisaged once the key drivers would have been captured but brisKar.

Overall, both FLORSYS and brisKar examples demonstrated that the assessemnt of agronomic traits such as HT or Bt should consider the specific receiving environments in which they might be introduced as

their positive or negative impacts highly depend on them. Due to the high diversity of management systems, ERA and PMEM need to be closely articulated and exposure models such as FLORSYS and brisKar are useful tools to implement a flexible and dynamic assessment and management of GM crops. Their practical use still need targeted improvement to fit end-user needs and constraints. This implies full commitment and collaboration of risk managers.

References :

Angevin, F., Klein, E.K., Choimet, C., Gauffreteau, A., Lavigne, C., Messean, A., Meynard, J.M., Messéan, A., 2008. Modelling impacts of cropping systems and climate on maize cross-pollination in agricultural landscapes: The MAPOD model. *Eur. J. Agron.* 28, 471–484.

Bürger, J., Granger, S., Guyot, S. H. M., Messéan, A., Colbach, N., 2015. Simulation study of the impact of changed cropping practices in conventional and GM maize on weeds and associated biodiversity. *Agricultural Systems* 137, 51-63.

Colbach, N., Collard, A., Guyot, S.H.M., Mézière, D., Munier-Jolain, N.M., 2014b. Assessing innovative sowing patterns for integrated weed management with a 3D crop-weed competition model. *Eur. J. Agron.* 53, 74-89.

EFSA Panel on Genetically Modified Organisms (GMO) 2010. Guidance on the environmental risk assessment of genetically modified plants. *The EFSA Journal* 1879, 1-111.

EFSA Panel on Genetically Modified Organisms (GMO), 2011. Scientific Opinion on guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. *EFSA Journal* 2011;9(8):2316. [40 pp.] doi:10.2903/j.efsa.2011.2316

Holst, N., A. Lang, G.L. Lövei, and M. Otto (2013). Increased mortality is predicted of inachis io larvae caused by Bt-maize pollen in European farmland. *Ecological Modelling* 250, 126-133.

Lamichhane J.-R., Devos Y., Beckie H., Owen M., Tillie P., Messéan A., Kudsk P. (2016). Integrated weed management systems with herbicide-tolerant crops in the European Union: lessons learned from overseas. *Critical Reviews in Biotechnology*, 2016.

Neve, P., Diggle, A.J., Smith, F.P., Powles, S.B., 2003. Simulating evolution of glyphosate resistance in *Lolium rigidum* I: population biology of a rare resistance trait. *Weed Res.* 43, 404-417.

Perry, J.N., Devos, Y., Arpaia, S., Bartsch, D., Ehlert, C., Gathmann, A., Hails, R.S., Hendriksen, N.B., Kiss, J., Messéan, A., Mestdagh, S., Neemann, G., Nuti, M., Sweet, J.B., Tebbe, C.C., 2012. Estimating the effects of Cry1F Bt-maize pollen on non-target Lepidoptera using a mathematical model of exposure. *Journal of Applied Ecology*, 49, 29-37.

Pidgeon J., May M., Perry J.-N., Poppy G.-M. (2007). Mitigation of indirect environmental effects of GM crops, *Proc. R. Soc. B* (2007) 274, 1475–1479 - doi:10.1098/rspb.2007.0401