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the Impacts of Genetically modified plants on Agro-ecosystems

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Principles for selecting field sites for ERA/PMEM

Towards a standardised matrix for applicants for field release of transgenic GM plants

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Contents

Preface

The current European regulation of genetically modified (GM) plants requires an Environmental Risk Assessment (ERA) before cultivation can be decided, and a Post Market Environmental Monitoring (PMEM) to detect, amongst others, any delayed or unanticipated adverse effect on the environment after the GM crop has been planted. To select field sites to perform the ERA one should consider 1. the crop plant, 2. the novel trait relating to its intended effect and phenotypic characteristics of the GM crop plant, and 3. the receiving environment related to the intended use of the GM crop (EFSA, 2010; EFSA, 2015). The PMEM consists of a case-specific monitoring to confirm assumptions about adverse effects and/or cover uncertainties identified in the ERA and a general surveillance to detect any unanticipated adverse effects associated with the release and management of the GM crop (EFSA, 2011). Because of their different scopes, selection of suitable field sites for either ERA or PMEM generally differs.

This report (AMIGA deliverable D2.3) describes the results of a study to further develop a guidance toolkit to support the selection of ERA sites, mostly for NTO-oriented field studies within receiving environments where the GM crop may be grown. This "toolkit" is meant to bring together the ideas and analyses concerning the selection of field sites for ERA from AMIGA into a practical line of thinking.

The "EFSA guidance document on the agronomic and phenotypic characterisation of genetically modified plants" (EFSA, 2015) provides recommendations on the selection of sites for the agronomic and phenotypic characterisation of GM plants, which is part of the comparative analysis between the GM plant and its non-GM comparator. Although this guidance document has a different purpose, it is taken into account in this document.

1. Approach

To support the selection of ERA sites within the receiving environments in which the GM crop is expected to be released, we base our selection procedure on the steps formulated in the EFSA guidance document on the agronomic and phenotypic characterisation of genetically modified plants (EFSA, 2015). These steps are: 1. plant; 2. plant x trait, and 3. plant x trait x environment.

In the first step the present potential and actual distribution range of the crop has to be identified. The second step identifies possible changes in cultivation areas and production systems because of the new trait. The third step involves the selection of environments representative for testing endpoints that are proper indicators for the environmental issues of concern. For ERA in general, these concerns are persistence and invasiveness of the GM plant or its compatible relatives, plant-to-micro-organism transfer, interaction of the GM plant with target organisms and with non-target organisms, impact of the specific cultivation (including management and harvesting techniques and considerations of the production systems and the receiving environments), effects on biochemical processes and effects on human and animal health (EFSA, 2010). It should be stressed that, depending on the area of concern, site selection may differ. Therefore, although some principles would equally apply to the other areas of concern, this report specifically addresses NTO field studies.

We will first bring together ideas and insights with respect to the selection of field sites where GM-crops and their non-GM crops to be compared with will be grown for ERA, considering the region-specific production systems, ecosystem services and protection goals in the anticipated receiving environment. We also included the assessment of ecosystem services, which are crucial for sustainable production, and may influence management towards the protection goals in the receiving environment. These aspects will be structured by means of tables and a flow diagram. The resulting selection toolkit will be evaluated by using data derived from the AMIGA field studies (see Deliverable 2.4).

2. Exploring the expected actual cropping area in a mechanistic approach

After identification of the potential area where the crop could be cultivated (based on existing agricultural data bases, expert opinions and/or modelling), possible effects of the genetically modified plant on the growth defining, limiting and/or reducing factors need to be assessed. For example, drought resistance traits can significantly widen the cropping area, whereas traits such as pest resistance can also widen the possible cropping area, but will probably do that in another way. Selection of ERA sites should anticipate on such possible alterations.

Therefore, in order to select relevant sites, the impact of the intended GM trait on the distribution of the crop needs to be identified. Here we follow a production-ecological approach (Van Ittersum & Rabbinge 1997) in which factors are distinguished that determine the photosynthesis, biomass production and yield, given that all other requirements are optimally available (factors category A), abiotic factors that determine the crop growth (category B) and finally factors (category C) that determine growth considering biotic interactions (Annex 1, box 1):

A) Growth defining factors; CO_2, radiation, and temperature requirements, and crop characteristics such as crop canopy architecture, physiology and phenology

B) Growth limiting abiotic factors; water and nutrient requirements

C) Growth reducing biotic factors; weeds, pests and diseases.

These factors identify a set of conditions required for crop growth and therefore determine the area, in which the GM crop can be expected to be grown after market introduction (Annex 1, box 2). These factors also will determine the agronomical practices required to grow the GM crop, and the production system according which the GM crop will be grown.

These defining, limiting and reducing growth factors help to structure and further substantiate the approach described in the EFSA guidance document on the agronomic and phenotypic characterisation of genetically modified plants (EFSA 2015) which suggests to start with a map depicting the current cropping area of the crop of interest in general, in order to set the rough boundaries of the area in which sites should be selected. In the second step in that document, the authors suggest the use of other dimensions, such as maturity groups (depending on the cultivar phenology), to further characterise possible growing areas. These two steps encompass the growth defining factors (category A). The third step the guidance document mentions is the creation of maps considering factors which will determine the possible growing area, such as water and nutrient availability (category B) or weeds, pests and diseases (category C).

We propose to start with the aforementioned factors that define growth, followed by growth limiting and finally growth reducing factors because this sequence attunes well with the current approaches in mechanistic modelling of crop growth. For a wide range of crops, crop growth models are available (see, e.g. http://models.pps.wur.nl/models). These models can help determine the possible cropping area of a crop based on a number of growth defining and, in specific cases, growth limiting factors. These models use the linear relationship between biomass production and the amount of radiation intercepted by the crop canopy to calculate the potential yield. The models use algorithms in which phenological development in relation to temperature, dry matter partitioning among the crop organs (harvestable product) and leaf area development until crop closure are accounted for (Van Ittersum et al., 2003). Climatological data (radiance, temperature, day length) of potential cropping areas as input to the model, allow predicting the potential production of the crop of interest in those areas (http://www.yieldgap.org).

The EFSA guidance document (2015) states that although the cultivation of a given crop line will be optimal in its maturity zone, it would be possible to grow it in adjacent zones. The mechanistic crop growth models are helpful to indicate reasonable, practically relevant boundaries to map such, e.g adjacent zones where the crop might yet be cultivated after market introduction.

Geographical information on the factors of categories B and C may similarly be explored to be better able to reflect the “different meteorological and agronomic conditions under which the crop is to be grown” as defined in Implementing Regulation (EU) No 503/2013 (cf. EFSA, 2015)

These models have already been widely used in practice to specify potential and actual growing areas. See for a detailed description of applications Van Ittersum et al (2003), Van Ittersum et al (2013), and Boogaard et al (2013). These models are currently applied to develop the Global Yield Gap Atlas for the major crops (http://www.yieldgap.org).

3. Relevant biogeographical areas

After exploring the expected actual cropping areas, including the possible changes in these as a result of the introduction of a certain new crop trait, we propose the use of biogeographical regions when selecting representative sites (http://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-1 (access: 6 November 2015), (Annex 1, box 3). To ensure that the resulting cropping areas fit into an institutional framework across Europe, we propose to overlay these zones with the potential cropping area of the plant containing the trait of interest. The advantage of this is that it makes possible to refer to other EU-policy related environmental impact assessments such as studies required to collect input data for registration of crop protection agents.

Jänsch et al. (2011) reviewed classification approaches of receiving environments with respect to ERA on non-target invertebrates in Europe. The purpose of ERA is not just about impacts on NTOs (see chapter 1). However, here we endorse their approach as potentially suitable for the wider ERA purpose. They stated that a suitable classification of biogeographical regions should be both ecologically relevant (e.g., close relationship between protection goals and receiving environment) and feasible (due to limited time and resources for ERA). The most suitable approach appeared to be the definition of ecological regions by the European Environment Agency EEA (2011) (http://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-1), because this approach:
- covers a manageable number of regions, i.e. 9 biogeographical zones,
- is developed on the ground of potential natural vegetation, thus indirectly on climate,
- is already recommended and used by the European Food Safety Authority EFSA (EFSA (2010).

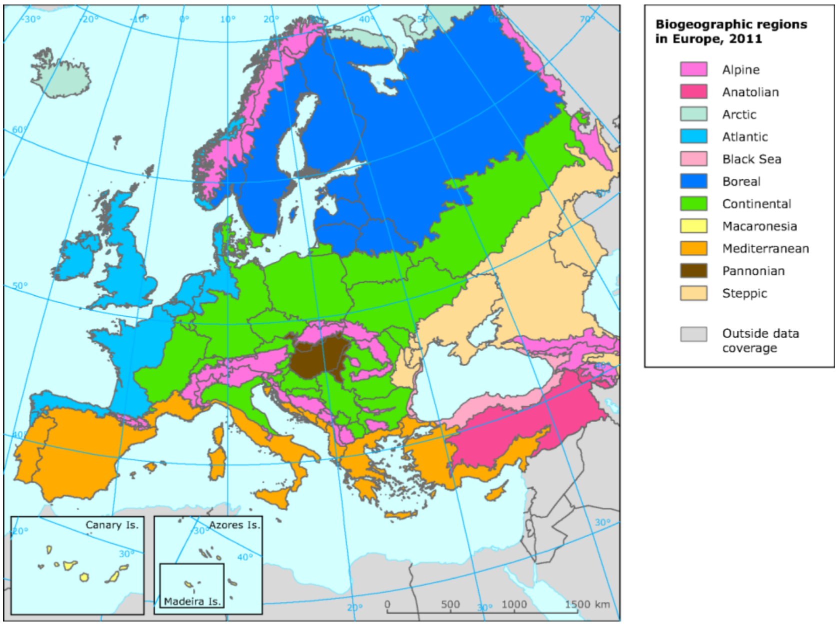

Fig 1 biogeographical regions in Europe, http://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-1 (access: 6 November 2015).

Jänsch et al. (2011) compared the EEA concept to the approaches of EPPO, Focus, ELCE and DMEER. EPPO zones (European and Mediterranean Plant Protection Organization), consists of only four different biogeographical zones, which may be too coarse, and the zones are defined by member state borders (EPPO, 2005). The Focus-concept does not cover the actual area of the EU, and does not take into account vegetation data (Van der Linden et al., 1997). The ecological land classification of Europe ELCE defined numerous eco-regions (40 – 200) which are too many for ERA purposes to be practicable (Hornsmann et al., 2008; Weustermann et al., 2009).

A digital map of European regions (DMEER) was developed by the Topic Centre on Nature Protection and Biodiversity (ETC/BD) defining more than 70 eco-regions which is not practicable (EEA, 2003).

Given the result of the review by Jänsch et al. (2011), we conclude that the EEA classification of Biogeographic zones is the most suitable for specifying ERA sites in Europe. These nine zones are: *Alpine, Atlantic, Black sea, Boreal, Continental, Macaronesia, Mediterranean, Pannonian, and Steppic* (excluding the *Arctic*, which is not relevant for crop growing) (Figure 1). In fact, AMIGA field work indicated meaningful regional differences in e.g. the species pool and diversity in Lepidoptera, one of the important NTO groups (see Annex 2).

4. Considering agronomic practices for envisioned crop in site selection within the biogeographic areas

After the identification of (parts of) the biogeographical regions in which the crop can be grown – either or not supported by means of model studies - , the same factors used to determine the potential and actual cropping area can also be used to identify the possible changes in agronomical practices. For instance, a drought resistance trait will change the water requirements of a crop and potentially expand the area within the biogeographical regions in which the crop is grown, or even expand the cropping area to other, more water limited, biogeographical regions. This, in turn, will potentially alter the agronomic practices in the biogeographical regions involved. Crop rotation in the regions may be wider or smaller, depending on how well the crop fits the current rotation in the area, the field sizes used may increase due to a reduced need for water management infrastructure, margin number and sizes may potentially change due to changes in field sizes, and so on. The result of this approach is an overview of possible sites in which the GM plant of interest may be cultivated and the possible changes in the agronomic practices as a result of that GM plant (Annex 1, box 4). This information may be captured in tables (e.g. Table 1 and Table 2 below) and in maps as suggested by the EFSA (2015) and is relevant for selecting the right, suitable fields for performing ERA.

Table 1 Changes in crop characteristics defining crop distribution over biogeographical regions and changes in agronomical practices

Main criteria	Factor	Characteristic	Affected biogeographical region	Affected agronomical practice
Changes in defining factors?	Changes in temperature requirements?	e.g. faster development rate	Alpine/ Atlantic/ Black Sea/ Boreal/ Continental/ Macaronesian/Mediterranean / Pannonian/ steppic	rotation/land use (field size and shape, use of margins)/tillage/fertilization/ planting density/planting arrangement/ sowing date/ pest and disease control/weed control/ water management and irrigation/harvest methodology
	Changes in photosynthetic active radiation requirements?			
	Changes in CO_2 requirements?			
	Changes in canopy architecture?			
	Changes in crop physiology?			
Changes in limiting factors?	Changes in water requirements?			
	Changes in nutrient requirements?			

Changes in reducing factors?	Changes in disease resistance/tolerance?	
	Changes in pest resistance/tolerance?	
	Changes in seed banks?	
	Changes in herbicide resistance/ general weed control?	

Table 2. Altered agronomic practices after identified changes in the characteristics of the receiving environment

Altered agronomic practice	Environmental characteristic impacted
Crops? **Rotation altered?**	landscape structure/mean field size/shape/ elements (hedge rows, margins)/protection goals related to interacting with crop/ Natura 2000 reserves/ surface water/water ways

Fertilization?

Planting density/arrangement?

Sowing/harvest date?

pest and disease control?

weed control?

water management and irrigation?

harvest methodology?

farming practices (e.g. field sizes)?

5. Considering relevant ecological functions and local protection goals per biogeographical area

Within relevant biogeographical regions, the altered agronomic practices (Annex 1, box 4; Table 2) may also affect the relationship with landscape elements such as field margins, hedgerows and/or waterways. The representativeness of field sites with regard to the receiving environment will also depend on the average landscape structure in the region and how these will be impacted. At a regional level specific protection goals may be of relevance and these need to be taken into account (see also case example 1 in Annex 2). Some of the protection goals are the result of European regulation (e.g. Natura 2000), and are relatively easily identified. However, different legislative and non-legislative sources at several administrative levels are used to protect the environment in a specific area. These levels comprise the international, subnational and regional administrative levels.

Another difficulty is that the formulated environmental protection goals vary from specific species, groups of organisms, to abiotic and biotic functions and services. To take these protection goals into account, within Work package 2 task 1 of the AMIGA project, a case-by-case method has been presented that helps select the species relevant to environmental risk assessment at the regional level (Miklau et al 2014). We propose to include the case-by-case method presented by Miklau et al (2014) after the identification of the relevant biogeographical areas (Annex 1, box 5). Miklau et al. (2014) used FFH species (Lepidoptera) as a case example on how to select harmonized protection goals at EU level for consideration in the ERA of GMOs. Start of the selection procedure was the evaluation of the distribution of the respective species in different biogeographic regions. Due to their endangerment and distribution over Europe a number of plant and animal species have been adopted into the annexes of the Habitats Directive. They are called “FFH species”,

The next step was to evaluate whether there is a possible overlap of the occurrence of the selected Lepidoptera with maize growing regions by a GIS-based analysis. In the present analysis it was shown that certain Lepidoptera may be more relevant for consideration in the ERA of GMOs than others due to the concentration of maize in their distribution area. In addition, to determine the spatial exposure we suggest to also consider temporal exposure of the species to GM crop cultivation if deemed necessary (see case example 1 in Annex 2).

The existing baseline diversity and species assemblages are also relevant in further directing the selection of experimental sites. One recurring question is how diverse the regions are, to require detailed ecological studies separately for receiving environments. The practical approach taken by AMIGA consists of reviewing existing literature and faunal data bases, integrate existing data with recent surveys conducted by AMIGA partners in the selected regions, evaluate differences in order to come with a zoning approach defined on the base of existing biodiversity. In general, our limited baseline surveys did not indicate a drastic difference of the current arthropod assemblages with respect to existing data (Lövei et al. 2014), but we filled some spatial gaps, and found a general northward shift of more "southern" organisms.

Information on sampling methods, time spans of collections and class and order distribution of arthropod predators and parasitoids in maize and potato crop according to their location in Europe have been extracted from an European arthropod database. The database contains 5499 records of 1679 species from maize and 2637 records of 793 species from potato. These records come from 31 countries, with the highest numbers from Germany. Fifteen methods have been used to collect these data, with pitfall traps being the most frequent. The most common predators include predatory beetles and spiders in both crops, with the share of beetles higher in maize than in potato. Parasitic Hymenoptera dominate the parasitoid guild in both crops. Sampling duration, composition by families and species, and methods summary provide useful guidelines about the methods to be tried for their potential as monitoring tools.

The amount of predator records in maize is 3044 including 828 different species. The continental region shows the highest amount of records followed by the Pannonian and the Atlantic regions, and these are the main maize growing regions in Europe. The most common orders found in nearly all regions are the Coleoptera belonging to the class of Insecta and the Araneae belonging to the class of Arachnida. The amount of parasitoid records in maize crop is 247 including 107 different species.

The amount of predator records in potato is 746 including 314 different species. The continental region shows the highest number of records followed by the Atlantic region. The most common orders found in nearly all regions are Coleoptera. Spiders (Araneae) are common in the continental and the Pannonian regions. There are only 29 records of parasitoids, including 26 different species, which is a small amount of records to present a reliable picture of the parasitoids in potato.

In conclusion, the results of the AMIGA arthropod monitoring (e.g. in Task 2.2) so far and the availability of a robust method to measure insect predation intensity, suggest that assessments of beneficial ecological processes /ecosystem services do not require additional site selection criteria.

6. Considering impact on indicator species and ecosystem processes within biogeographical regions

The first steps were the identification of the potential cropping area of the current crop, and the envisioned changes in the cropping area due to the envisioned introduction of the specific trait of interest. This was followed by the determination of involved biogeographical regions and specific European, national and local protection goals within these regions.

The next step is to link the changing characteristics of a selection of representative receiving environments as identified by steps 1 to 5 (Annex 1, box 1 to 5), to the distribution of relevant target and non-target species, and processes and indicators that link to other concerns that must be addressed in an ERA (Annex 1, box 6) and thus include requirements to assess these concerns into the site selection process.

A good starting point for this task is a flow diagram for the complete planning of ERA field trials for non-target organisms, as developed by Hilbeck et al. (2014). The first step in their flow diagram is to identify the important ecological functions of the given cropping system (in our own approach relevant cropping systems that result from the Table 1 end 2 exercises) and receiving environments in which the GM crop is intended to be used. In their second step in Figure 2, Hilbeck et al. (2014) rank species and ecological functions. In case the contributing species are unknown only ecological processes need to be considered, e.g. functional processes in the soil. This second step may, indirectly or even in an iterative way, be relevant to the selection of ERA field sites, since for example the selection process of such site may depend on the actual geographical distribution of a certain highly ranked non-target key species.

If from desk studies the ERA starts with, it is concluded that for the specific plant with the envisioned GM trait, the fauna relevant to the agroecosystem in different regions is known (from the database and from the additional outcomes of AMIGA), most appropriate endpoints can be selected to tackle NTO issues in regions previously screened according to steps 1 and 2. According to that we then select and consider assessment endpoints following the flow diagram and this will enable to decide how many and where do we need them

Once based on the aforementioned theoretical exploration test organisms and processes are selected, the distribution of the indicator species within the biogeographical regions can be used to select relevant fields (Annex 1, box 7).

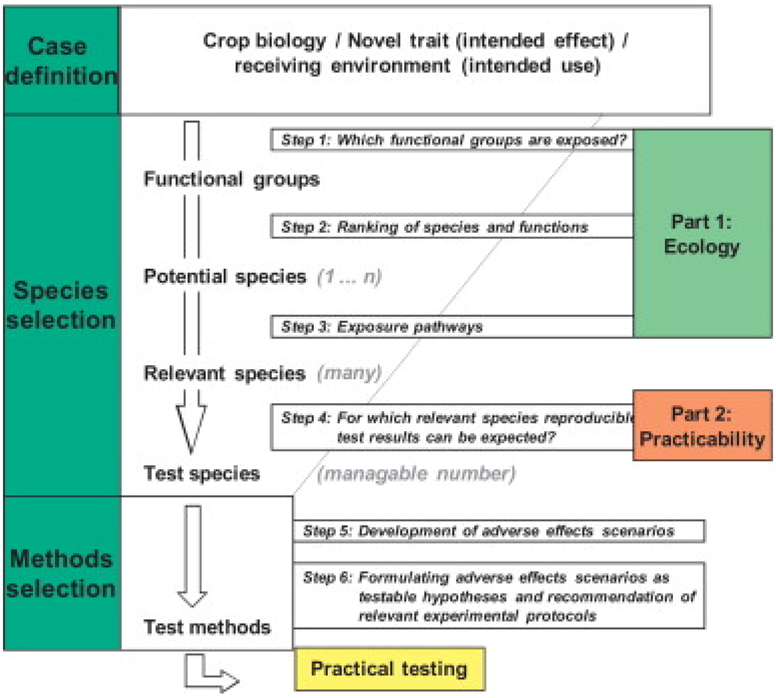

Fig. 2 Flow diagram for the complete planning of ERA field trials (Hilbeck et al., 2014). "Practical testing" at the end of the flow chart involves field site selection (see Annex 1).

7. Discussion with respect to selection of ERA sites

We have drawn up a standardized matrix for selecting field sites for ERA. For ERA in general, environmental issues of concerns are persistence and invasiveness of the GM plant or its compatible relatives, plant-to-micro-organism transfer, interaction of the GM plant with target organisms and with non-target organisms, impact of the specific cultivation (including management and harvesting techniques and considerations of the production systems and the receiving environments), effects on biochemical processes and effects on human and animal health (EFSA, 2010). We restrict ourselves to selection of those sites where for ERA purpose GM and comparator non-GM crops will be grown to study impacts on non-target organisms under representative, i.e. expected actual, growing conditions.

The matrix includes additions to the EFSA approach in the use of some climatological aspects and the use of crop growth models. The use of these crop growth models is not essential in case regional data are available about crop distribution. For Europe these distribution data are available for most crops, e.g. for maize. However, if one may expect that the envisioned introduction of the GM trait of interest may lead to differences in potential and actual cropping area compared to the non-GM crop variant, the proposed mechanistic modelling will be of help to describe, and if possible narrow, the borders of the region within Europe where this new crop may be grown with the best available data and insights.

Two case studies have shown that the suggested approach is in general feasible for selecting sites for ERA (Deliverable 2.4).

It is evident that site selection has to be conducted case-specifically as the given crop and the selected protection goals strongly affect the outcome of the evaluation and choice of field locations. In case of non-target organisms a prior exposure analysis, both spatially and temporarily, is a determinative component. Although a legal GMO approval is European-wide, site selection should be adapted to the bio-geographical regions in order to account for regional characteristics, e.g. farming practices and protection statuses. In particular cases it might be difficult if not impossible to precisely predict changes in agricultural management or a shift in cultivation areas of crops following the introduction of a given GM crop, even after explorations with mechanistic crop growth models. Also variability within bio-geographical regions might be difficult to consider, e.g. with respect to impact on certain specific protection goals at the national level. If there is a well-argued view, i.e. an identified reason, about this prediction difficulty, e.g. developed after the performances of the ERA, an adjusted site selection for part of the PMEM (i.e. the case specific monitoring) may compensate for this (see results from WP7).

To guide the design of ERA field trials as above described and restricted to, specific methods for power analysis for statistical tests based on field trial count data have been developed in the AMIGA project, as will be fully reported in Deliverable 9.4 (see also Goedhart et al. 2014, van der Voet and Goedhart 2015, Kruisselbrink et al. in prep.). This has resulted in the availability of publically available software for this purpose in the form of the AMIGA Power Analysis tool (Deliverable 9.5, Kruisselbrink et al. 2016). For the purpose of the experimental design of ERA NTO field trials in Europe the methods and software can be used in two scenarios.

1. The first scenario is a single-environment field trial (i.e., a field trial in single location/year) in which the aim is to determine the number of replicates needed to obtain sufficient statistical power. In the site selection process, especially while checking whether enough homogenous plot area will be available for experimental design, this scenario is relevant to indicate the required minimum size of a site where the experiment will be carried out. The more replicates needed, the larger the field should be. Note that the field size might be restricted due to a maximum area a non-authorised GM crop might be grown according the permit given by the authorities.

2. The second scenario is a multi-environment field trial in which the aim is to determine the number of environments (i.e., location/year combinations) in order to obtain sufficient statistical power. Here we assume a fixed design at each environment (e.g. 4 replicates of GM and non-GM comparator), and calculate the number of replicates at this higher level (i.e. the location/year combination) in the power analysis. This scenario is relevant for selecting ERA field sizes while it indicates based on statistical principles how many sites within a biogeographical region should be selected

A case study based on historical data provided by Prasifka et al. (2008) has been performed in WP 9 to further elaborate on these two scenarios and will be reported later (Kruisselbrink et al, in prep.).

A general issue for developing criteria to interpret possible beneficial and adverse effects on key processes and species at the selected sites of ERA field trials is that statistical power can only be evaluated given specified relevant effect sizes. Without specification of LoCs (or targeted effect sizes) by experts in the field no power analysis can be performed. In AMIGA tentative limits of concern have been set as pragmatic triggers for further consideration. E.g. for non-target arthropods and soil organisms preliminary LoCs were set at two-fold increases or decreases with respect to the comparator abundance provided this abundance is not too low. A further elaboration of the application of limits of concern, illustrated with case studies from the AMIGA project, is given in the statistical protocol (Deliverable 9.4). Different interpretations of LoC are discussed in Deliverable 9.6.

In our approach we restricted ourselves to selection of sites for NTO field trials. For ERA in general, other concerns that may need to be investigated are persistence and invasiveness of the GM plant or its compatible relatives, plant-to-micro-organism transfer, interaction of the GM plant with target organisms, impact of the specific cultivation (including management and harvesting techniques and considerations of the production systems and the receiving environments), effects on biochemical processes and effects on human and animal health (EFSA, 2010). A case-wise theoretical exploration exercise could be carried out to indicate which ERA concerns might be impacted given the envisioned introduction of the specific GM trait of interest. The question to be answered is whether requirements for a robust assessment of features and processes related to these concerns will differ for different GM traits and whether this will influence the ERA site selection. Within the scope of the current work package task no general criteria could be developed, given the broad range of traits that might need to be considered in novel breeding programmes in which GM is being used.

All the described criteria, however, were considered in the planning of experimental field work during the projects' activities and this constitutes also the base for building the AMIGA network of field sites which is now available for promoting possible future activities.

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Annex 1. Flow diagram field selection