

REVIEW

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Overview of principles and implementations to deal with spatial issues in monitoring environmental effects of genetically modified organisms

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Abstract

The approval of genetically modified organisms [GMO] for deliberate release and placing on the market requires GMO environmental risk assessment [ERA] and GMO environmental monitoring [EM]. Both GMO ERA and GMO EM are still under discussion. The goal of this article is, firstly, to analyse principles of GMO EM as published in the Association of German Engineers [VDI] Guideline 4330 Part 1, focusing on the characterisation of the receiving environment affected by GMO cultivation and the representativeness of GMO EM to assess large-scale implications of GMO cultivation. Secondly, the article introduces measures to meet these issues by the use of map data and statistics within a geographical information system [GIS]. Finally, three case studies exemplify the application of data and methods. To deal with spatial issues of GMO EM as outlined in the VDI Guideline 4330 Part 1, a GIS-based approach is presented. It relies on both spatial data collected from several sources which were derived from sample point data and geostatistical and multivariate statistical methods within a GIS environment. Data used for describing the receiving environment and for planning and evaluating monitoring schemes comprise information about land use, climate, phenology, soil coverage, species distribution and ecoregions. The case studies deal with (1) ecological land classification for characterisation of GMO-receiving environments and representative EM, (2) selection of representative sites for modelling GMO dispersal, and (3) delineation and mapping of segregation distances. Even a systematic and stepwise-structured risk assessment cannot cover all risk relevant questions, especially large-scale, long-term and combinatory effects which may not occur before the conventional application of the respective GMO. Hence, GMO EM is crucial to deal with unanticipated and undesirable effects. The article gives an overview of a GIS implementation and relevant geodata promoting GMO EM.

Background

In the European Union [EU], the release of genetically modified organisms [GMO] into the environment is regulated by EU Directive 2001/18/EC [1]. Accordingly, post-market environmental monitoring of genetically modified plants [GMP EM] has to be implemented to detect and prevent adverse effects on human health and the environment. However, no general strategies for GMP EM have been established so far. In Germany, one EM strategy discussed is the Guideline 4330 Part 1

published by the Association of German Engineers [VDI] [2]. It applies to the monitoring of ecological effects of GMP, but does not address possible effects of GMP on human health. Contrary to the directive of the European Community [1] and the study of Sanvido et al. [3], the guideline of the VDI [2] does not differentiate between case-specific monitoring [CSM] and general surveillance [GS]. CSM should focus on anticipated effects of a specific GMP based on pre-market risk assessment, whereas GS is designed to detect unanticipated adverse effects which were not covered by risk assessment comprising, for instance, cumulative and long-term effects.

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The VDI [2] covers *ecological effects* of GMP encompassing direct, indirect, immediate and delayed as well as cumulative long-term effects. Environmental effects of GMP can occur on several *levels of ecological organization* in terms of structures and underlying functions, and correlated *levels of time and space* which have to be covered by GMP EM [4]. The VDI [2] provides planning and implementation criteria for GMP EM and forms the framework for technical instructions with respect to the levels mentioned above, *items to be protected*, *protection targets* and *checkpoints*. GMP EM should allow evaluating the condition of the items to be protected and to track the accomplishment of protection targets. The required parameters have to be collected using validated and standardised methods.

Since protection targets and checkpoints may not only be influenced by GMP, it is necessary to differentiate between GMP-related effects and those that are not related to GMP. Accordingly, data on items to be protected and protection targets without influence of GMP must also be compiled. To reach this requirement, temporal and spatial comparisons are needed: The environmental baseline status prior to the introduction of GMP has to be compared to the situation after GMP release regarding the selected checkpoints. The reliability of reference data depends on the period within the reference conditions were monitored before introducing GMP (*temporal comparison*). Additionally, GMP-free regions have to be compared to those where GMP are cultivated. This requires a reference system where both conditions are monitored simultaneously (*spatial comparison*). Reference sites should be as similar as possible to GMP-influenced areas considering the receiving agricultural environment. GMP areas and reference areas, however, can also be subjected to changes that are not caused by GMP. Thus, a thorough selection of monitoring areas is essential for monitoring potential ecological effects due to GMP cultivation. Besides the GMP fields and their surroundings, representative types of ecosystems that will potentially be affected should be considered as well. EM areas should be selected using a statistically substantiated procedure according to technically suitable representation criteria. The EM areas should be linked to other appropriate EM networks. In the long term, spatial rearrangement of EM areas is necessary regarding new effect relationships and spatial arrangement of land use patterns.

The GMP EM measuring data should be analysed on the basis of metadata describing them and by suitable (geo)statistical procedures. The documentation of measured variables, methods, survey intervals and areas must be carried out according to standard methods and using a main meta-database or several interrelated databases. Meta-databases should help evaluate on to what

extent the data records can be compared with one another for assessment.

Based on the basic considerations as laid down in the guideline of the VDI [2] and summarised above, some research projects aimed at dealing with GMP EM at a landscape level and at developing techniques for supporting the application of the respective EM strategies. In the following, we refer to some of the respective methods and results and, thereby, concentrate on the setting in Germany as an example.

Methods and data

The following sections contain an overview of procedures implemented in a *geographic information system [GIS]* including *geostatistics*, *multivariate statistics* and *geodata* to (1) characterize the GMP-receiving environment, (2) to assess the spatial representativeness of GMP EM sites and (3) to assess large-scale and long-term effects of GMP cultivation. In Germany, several research projects dealt with these issues, and some of the methods applied and results achieved are outlined. It is shown that geodata are useful to describe the receiving environment in the near and far vicinities of GMP fields. Statistical analyses and classification of geodata are presented which serve to derive ecoregions, e.g. climatic and agricultural patterns and, thereby, help for assessing the representativeness of running or planned GMP EM sites and for investigating adverse ecological effects of GMP release on different spatial scales and for different agricultural regimes [5-11].

Geostatistics is a point-pattern analysis that generates surface predictions from data points. This relies on investigating and modelling the spatial autocorrelation among sample data by variogram analysis. In order to apply kriging for interpolation, it is necessary to adapt a defined variogram model to the experimental variogram. Based on the variogram model, several kriging methods can be used for spatial predictions which finally are mapped [12]. For the interpretation of the kriging estimations, a cross-validation has to be performed.

Multivariate statistics such as cluster analysis or tree-based models, two of them are the classification and regression trees [CART] and chi-squared automatic interaction detection (CHAID), serve to spatially differentiate the multiple relationships between geodata stored in a GIS. Based on these relations, predictions in time and space become possible as well as the characterisation of the receiving environment in terms of ecoregions [13-18]. In the context of GMP dispersal, *cluster analysis* can be used to integrate measurement data from different meteorological networks with different coverage in a GIS environment for defining representative climatic regions. Climatic regions together with an ecological land classification were used to stratify the

receiving environment in order to select a representative number of sites for modelling the GMP dispersal [10].

Geodata on meteorology, land use, local biodiversity and agricultural management schemes are needed for monitoring and modelling dispersal and persistence of GMP as well as planning GMP EM with respect to coexistence issues in agricultural landscapes. The data described in the following have been collected from several sources or have been calculated from sample point data by the use of the above mentioned statistical methods in a GIS environment described by Kleppin et al. [19].

Land use data can be obtained from either satellite images, GIS data collected during field experiments, cadastral surveys provided by local land registries, vertical air photographs or the Common Agricultural Policy notifications, each type of source being used at different scales and consequently provide different spatial and semantic resolutions. To some extent, data on field geometries providing detailed information on agricultural land use can be obtained from the Integrated Administration and Control System (InVeKoS) database, which is an important tool for the EU member states to regulate agricultural subsidies. In fact, due to legal restrictions and inconsistencies in data harmonisation which is due to federal responsibilities, this dataset is not available for public use [9]. Based on satellite images, data on land use patterns are offered by the European Topic Centre on Land Use and Spatial Information [20], where the distribution of the CORINE Landcover maps is administrated [21]. Data on the cultivation of crops in Europe are available at the Statistical Office of the European Communities [22], which offers data on various topics, among of which is also agriculture. The main cropping areas of oilseed rape are located in northeast Germany as well as in the Alsace in France. In these regions, oilseed rape is cultivated on up to 25% of the arable land. Due to the increased cultivation of energy plants, it can be assumed that the cultivation of maize (biogas) and oilseed rape (biodiesel) will be intensified in the future. For Germany, it can be stated that in 2007, there was an increase in maize cultivation of 9.6% and of 8.8% for oilseed rape cultivation compared to those in 2006.

For large-scale analyses of GMP impacts, *meteorological data* are needed. These are, for example, data on precipitation, air temperature, sunshine duration, the number of frost days and wind conditions. Climate affects the growth, persistence and dispersal of GM crops and their pollen and seeds. These data could be retrieved from meteorological stations, which are usually widespread in Europe. However, depending on the required climatic element (precipitation, air temperature, wind or solar radiation), the number of monitoring sites

and, thus, the validity of assumptions based on these data are different. For example, in France, the spatial density of monitoring sites collecting data on precipitation is two times more dense than on temperature, four times more than on wind and ten times more than on solar radiation. In Germany, the number of meteorological monitoring sites differs quite more. The German Weather Service operates about 4,400 precipitation sites, but only 660 stations for air temperature and 220 for solar radiation. Therefore, interpolations or extrapolations may be necessary, covering the whole territory of a country. For Europe, free datasets with a resolution of 10 arc min (approximately 20 × 20 km) are available at the Climatic Research Unit (CRU) [23,24]. For modelling the pollen transport, phenological data on the flowering of GM crops should be considered, too. It should be taken into account that global warming might have changed the temperature-induced beginning of rape and maize bloom [18,25]. Furthermore, modelling pollen dispersal requires data on wind regimes. The dynamics of pollen transport can be described by compiling and processing data on wind direction and velocity. The wind direction influences the transport direction of the pollen and, thus, potential areas of exposure. Given a constant emission rate, the wind velocity affects the range and the transport speed of airborne pollen and leads to a dilution (stretching); as with higher wind velocities, a larger air volume passes the source surface, and the concentration per unit volume is reduced [26].

Data on soil texture and soil types are available from the Food and Agriculture Organization [FAO]: (1) the Digital Soil Map of the World (about 10 × 10 km²) [27] and (2) the Harmonized World Soil Database (about 1 × 1 km²) [28]. Data on the potential natural vegetation which can be used for ecological land classification can be obtained from the Federal Agency of Nature Conservation (BfN) in Germany [29]. The potential natural vegetation [PNV] map stratifies Europe into more than 700 PNV units. The PNV can be defined as the vegetation that could be established without human interference under present climatic and soil conditions and is an integral indicator for the ecological conditions in terrestrial ecosystems [16].

For *biodiversity data* in the detection of adverse effects on biodiversity, a link between GMP and biodiversity monitoring is imperative [30,31]. It has to be expected that due to a large-scale commercial use of GMP, adverse effects on biodiversity become substantial. Biodiversity monitoring schemes could provide information on potential threats induced by GMP. For instance, biodiversity monitoring is able to detect the potential invasiveness of GM crops and the potentially enhanced mortality of non-target organisms, and it may also draw a more general picture on potential effects on the

countryside biodiversity. In Europe, several biodiversity monitoring networks exist due to the Convention on Biodiversity, which commits its signatory countries to identify and monitor national biodiversity. However, these monitoring networks are poorly connected, and data are usually available only on a local or national level [32], whereas the monitoring of birds and butterflies is well established over long periods in some European countries (e.g. > 30 years in the UK), allowing an assessment of changes at several trophic and geographical scales [33,34]; monitoring is not in the same quality established across taxonomic groups relevant for GMP EM. Only few larger scale monitoring schemes of plants exist [35]. As of September 2007, the EuMon database comprised 552 complete monitoring schemes covering approximately 4,000 species and 145 different habitat types and addresses of 239 monitoring coordinators and institutions. Furthermore, the database contains information on sampling methods.

Changes of biodiversity due to GMP cultivation must be extractable from the background noise of sampling variability and population fluctuations. This is only possible if a considerable amount of sites is frequently and accurately monitored and if reference areas, i.e. areas without potential influence of GMP, are monitored at the same time and with the same accuracy. Even though the EuMon database is the largest collection of metadata on biodiversity monitoring available, it is not comprehensive and might be confounded by biases in observation accuracy [36]. Besides the EuMon database, there are only few more data sources where information on biodiversity or distribution of plant species - that may, for instance, serve as crossbreeding partners of GMP - may be obtained. The Global Biodiversity Information Facility [37] enables free and open access to biodiversity data worldwide via the Internet to support sustainable development. An information system was built to allow the linkage of diverse data types from disparate sources, promoting capacity building and catalysing development of analytical tools for improved decision-making. A special application concerning forest data and the distribution of forest tree species is available through the European Forest Genetic Resources Programme [38], which is a collaborative programme among European countries to promote conservation and sustainable use of forest genetic resources. There is information available describing the spatial distribution of about 40 tree species occurring all over Europe. Data are stored as JPEG files but also as shape files for usage within a GIS environment. DIVA-GIS [39] is a free and open-source GIS to generate and analyse worldwide maps on species distribution data. DIVA-GIS was developed at the International Potato Center [40]. In Germany, the Federal Agency for Nature Conservation (BfN) maintains the

web application FloraWeb [41], where information on about 3,500 plant species are stored containing details on e.g. taxonomy, biology and spatial distribution of plants in Germany. An interactive web application illustrates the distribution of the PNV [29] in Germany. A Java applet allows mapping selected plant species in a spatial differentiation based on cadastre maps (scale 1 is 25,000; $\approx 11 \times 11 \text{ km}^2$).

A crucial problem for spatial analyses is the availability of *data on the distribution of present pests*. For the federal state of Brandenburg, there were data collected on a district level regarding the spatial distribution of the European corn borer (*Ostrinia nubilalis*) which was one reason for the introduction of Bt maize. Figure 1 depicts the distribution of the corn borer in the federal state of Brandenburg for the years 2005, 2006 and 2007.

Some of the data and methods presented above have been used in several case studies which have been conducted according to selected aspects of the VDI Guideline 4330 Part 1 [2]. Three of them are summarised below: (1) ecological land classification for characterisation of GMP-receiving environments and representative EM, (2) selection of representative sites for modelling GMP dispersal and (3) delineation and mapping of isolation zones.

Results and discussion

Case study 1: ecological land classification for characterisation of the GMP-receiving environment and implementation representative EM

The VDI Guideline 4330 Part 1 [2], the German Federal Nature Protection Law (Section 6 of the Bundesnaturschutzgesetz), the environmental monitoring concept of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety [42] as well as the preamble of the administrative agreement between the German government and the federal states on the exchange of environmental data specify the following targets that should be complied with when carrying out environmental monitoring: The monitoring should be coordinated and based on harmonised or standardised methods [43] so that the data can be compared and used for statistical analysis and modelling. The monitoring data should allow for spatial extrapolation in order to bridge geographical gaps and for supporting long-term research on environmental changes. The flow of data should be efficient, and the data should be available for scientists, especially for statistical testing of hypotheses and modelling data. The latter aspect also implies important technical issues because of the enormous amount of information and data collected. For example, environmental monitoring networks require information exchange, which has to be supported by an adequate and efficient information platform that handles

environment, but should also include regions with no or unknown GMP exposure as reference areas. On a case-by-case basis, depending on the GMP characteristics, the selected indicators, checkpoints and related analytical methods should consider different relevant spatial and temporal scales [2,6]. The number of monitoring sites and regions needs to be sufficient to support statistical analysis of results based on good scientific practice [47-49]. For each GMP monitoring, design and data analyses should be based on appropriate scales of space and time, and the quality and quantity of data should be representative and interpretable. Criteria for selecting monitoring sites and regions include representativeness of sites cultivated with specific GMP, with emphasis on regions repeatedly cultivated with GMP; representativeness of ecological regions containing the spectrum of relevant indicators; availability of sites already monitored within other environmental programmes; and areas with environmental conditions facilitating spread or survival of GMP [4,50].

In order to check the representativeness of existing EM networks which might be appropriate for EM GMP or for establishing specific EM GMP networks, ecoregionalisations are appropriate measures. For Europe and Germany, ecological land classifications were calculated by means of multivariate statistics and based on digital maps depicting the spatial patterns of ecologically relevant land characteristics. For both Germany and some federal states, ecoregions were calculated by applying CART and using surface maps on climate, altitude, soil and potential natural vegetation [6,16]. The resulting maps have a spatial resolution of 2×2 and 1×1 km². The land classification calculated for Europe by means of CART [13] subdivides the whole territory into ecoregions mapped in a grid with a cell size of about 20×20 km². Data used for calculating the ecoregions are maps on the PNV [29], on altitude (Global Land One-kilometer Base Elevation/GLOBE) [51], on soil texture (Digital Soil Map of the World/DSMW) [27] as well as on monthly averages on air temperature, sunshine duration, relative humidity and precipitation (Global Climate Dataset CL 2.0) [23]. The PNV was set as the target variable, whereas the above mentioned maps on altitude, soil texture and climate were chosen as predicting variables. In order to obtain a concise amount of ecoregions, the most detailed map depicting the spatial pattern of about 200 ecoregions was reduced to 40 ecoregions (Figure 2). Each of them can be described statistically and by the use of annual course diagrams and histograms as it is demonstrated for selected ecoregions (D_7 to D_22) in Figure 3.

Case study 2: selection of representative sites for modelling GMP dispersal

For modelling pollen dispersal of genetically modified oilseed rape [GM OSR], representative locations should be determined [5,10]. Accordingly, a method was

developed that includes both the determination of representative OSR locations for modelling the dispersal at a field scale and the subsequent generalisation of the modelling result to the landscape level at a regional scale (up-scaling). Accordingly, land characteristics which are relevant for dispersal and persistence of GM OSR were regionalised within a GIS environment. The beginning of flowering of OSR was mapped by means of geostatistics. The resulting maps were used to select satellite images for the detection of OSR fields and to determine the period for the individual-based modelling. The monthly means (1961 to 1990) of precipitation [P], air temperature [T] and sunshine duration [S] were regionalised by the Ward cluster analysis [52], which has a wide range of applications in landscape ecology [53-55]. The PTS clusters were combined to four climatic regions which, together with Ward clusters on wind speed and direction as well as with land use clusters (crop rotation and management) [56], enabled to define eight regions in Northern Germany with a maximum of internal homogeneity. A distinct meteorological station was selected to represent each of these regions. Data on wind speed and direction (hourly means), precipitation, sunshine and air temperature (daily) measured at that location were provided for modelling the growth, dispersal and persistence of GM OSR on selected fields on the local level [57]. Linking each of the modelled sites with a map on German ecoregions [16], which integrates the spatial patterns of soils, elevation, vegetation and climate, the modelling results were anticipated by analogy reasoning to be valid for all those ecoregions which are represented by the modelling sites and, thus, could be spatially generalised for up-scaling [58].

Case study 3: delineation and mapping of isolation zones

Concerning the protection of non-target organisms that might be harmed due to GMP cultivation, a methodology was developed to classify the susceptibility/sensitivity of nature reserves [NSG] in Germany as being part of the receiving environment that might be affected due to GMP cropping in their vicinity. Within the joint research project 'Recommendations for isolation distances concerning the cultivation of genetically modified plants in the neighbourhood of protected areas' funded by the Federal Agency for Nature Conservation (BfN), possible risks for biocoenoses in protected areas were evaluated as well as measures which could mitigate or hinder negative effects [7]. According to Section 23 of the German Federal Nature Protection Law [BNatSchG], NSG are to protect nature and landscape properties by preserving and developing as well as by re-establishing existing biotopes of wild and endangered species. According to Section 34a of the BNatSchG, the use of GMP has to be accompanied by an environmental

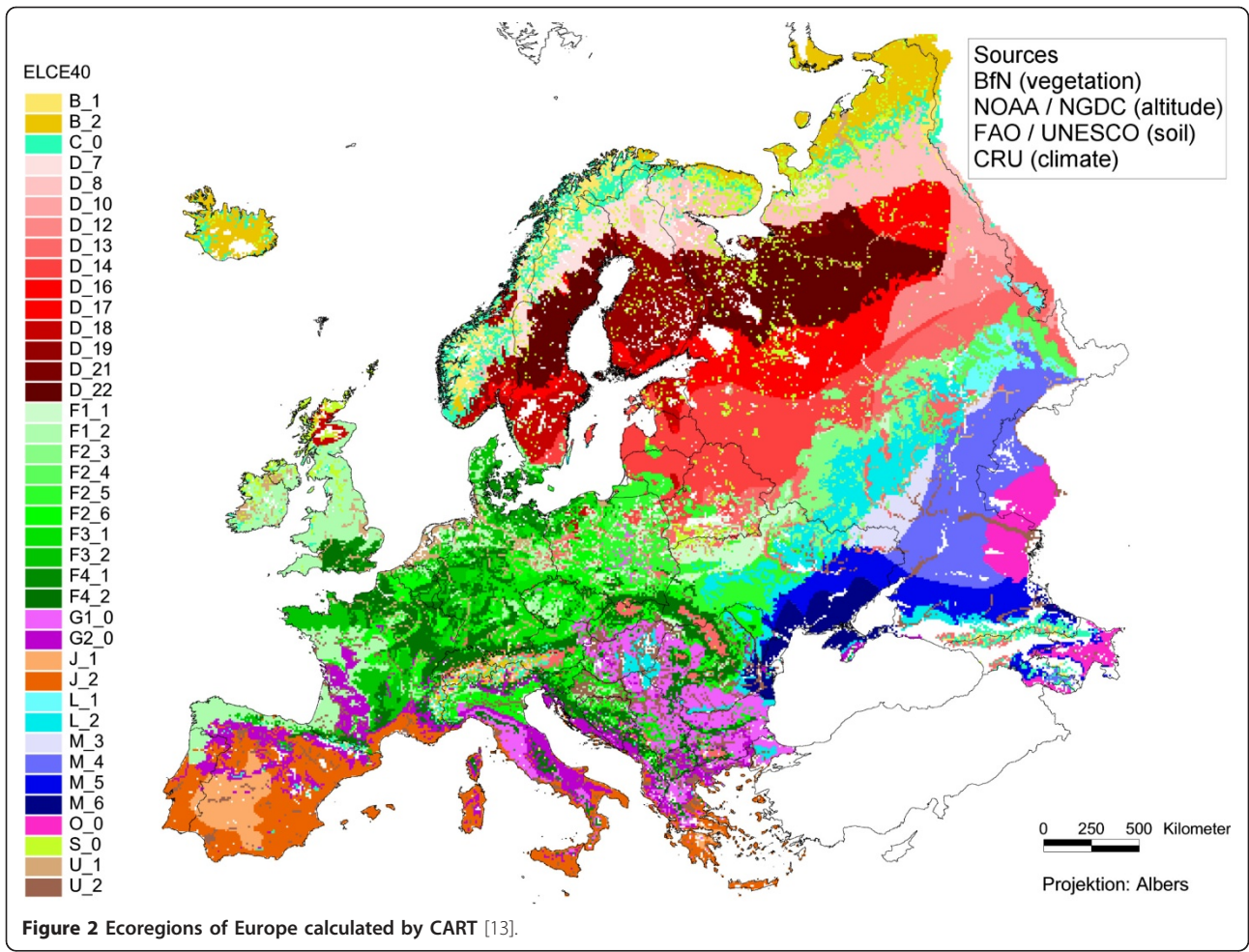


Figure 2 Ecoregions of Europe calculated by CART [13].

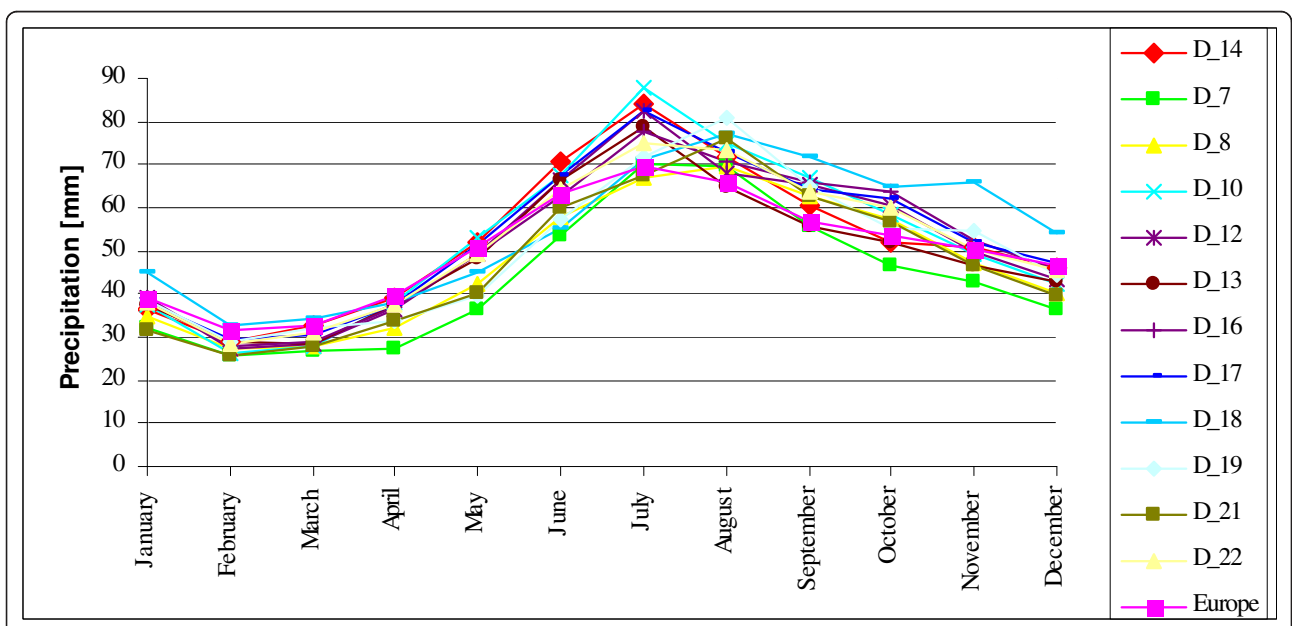


Figure 3 Annual course of precipitation (monthly means, 1961 to 1990) for some ecoregions in Europe [13].

impact analysis of possible risks like it has to be done in projects affecting the integrity of Flora-Fauna-Habitats (FFH) or European bird sanctuaries. In order to classify NSG according to their potential endangerment by GMP invasion, a methodology based on GIS techniques and statistical measures was developed. Additionally, it examined what implications would emerge when introducing different isolation distances concerning the cultivation of herbicide-resistant OSR and insect-resistant maize near protection areas [9]. Both should help in monitoring and modelling GMP impacts. Within a GIS environment, geometries of conservation areas, land use data (CORINE Landcover) [21], agricultural information on the district level (Easystat: Statistik Regional 1999) as well as a map of German ecoregions [16] were integrated. All NSG were classified with respect to geometric properties and different intensities of cultivation area in their vicinity. The classification was realised by calculating a geometric coefficient [GC] which described the ratio of the buffer zone and the NSG area in order to parameterize the risk of GMP invasion. The smaller and/or the narrower the NSG, the larger is the buffer zone, relatively, and the higher is the risk for GMP invasion. According to frequency analyses of the GC, three percentile classes (low/medium/high) were derived. The cultivation area of maize and OSR cropping in the buffer zone around the NSG was expressed by a cultivation coefficient [CC]. This was calculated by adding up the area of maize and OSR cropland within a radius of 800 m (maize) and 4,000 m (OSR) around the NSG. Considering GMP cultivation in the future, these GMP fields are likely to be located in those regions where cultivation of conventional crops already has taken place. On the other hand, conventional maize or OSR fields might act as stepping stones to establish transgenes from GMP fields far off by cross-breeding with conventional stands, volunteers or ferals. Again, three percentile classes were built by frequency analyses. They describe the spectrum from a low to a high cultivation intensity of maize or OSR in the neighbourhood of each of the 7,338 NSG in Germany. The combination of GC and CC resulted in a total of nine risk categories [RC], describing the potential risk of endangerment by GMP cultivation in the vicinity of NSG. Areas with the highest risk were grouped in RC 9: Here, those NSG were assembled showing the smallest acreage and the highest cultivation rate of the respective crop (maize, OSR) in the neighbourhood of the NSG. With a numerical proportion of 7%, those sites cover only 0.4% of the total area of all NSG. All NSG showing the highest CC values had a total proportion of 60% [9].

Conclusions

The GMP EM is an important element of the regulatory framework for GMO cultivation in Europe and

needs to be conducted according to scientifically sound methods and quality criteria to generate data which have to be robust and conclusive. The choice of parameters, methods and experimental designs of the locations and the timeframe for GMP EM needs to ensure that adverse effects of GMP and their use can be detected reliably and as early as possible. To reach this end, guidelines such as that of the VDI [2] are needed in attempting to harmonise and standardise the GMP EM design.

The VDI [2] recognizes that the environmental effects of GMP may vary with the characteristics of different receiving environments in terms of e.g. climate, soils, land use patterns or geographic distribution of wild relatives of certain GMP. Therefore, data derived by ERA or EM should be collected in those regions which are representative for respective ecological and agronomic characteristics which potentially could influence the spread and impacts of GMP. Thus, spatially differentiated monitoring schemes are needed, in particular with regard to biodiversity (e.g. non-target organisms) and ecological processes and functions (e.g. soil functions) in which these organisms are involved. However, access to relevant geodata is a prevalent problem. In this context, the EU directive Infrastructure for Spatial Information in Europe [INSPIRE^a] is an ambitious initiative to promote standardised data retrieval. In Germany, PortalU^b is a first step to achieve the INSPIRE goals. However, the problem so far is that only few geodata sets are available, less of them being appropriate for GMP EM use. Exposure assessment is crucial for GMP EM, aiming to assess whether relevant parameters, e.g. certain non-target species, have to be in focus in the course of the monitoring. In combination with an effect assessment, the exposure assessment allows the evaluation of species which may be at risk. Geodata, ecological land classification, spatial estimation and GIS techniques in combination with dynamic modelling are fundamental to address effects on a landscape scale and long-term implications, to analyse and evaluate the appropriateness of existing monitoring programs or data for GMP EM, to design adaptations or extensions of the scope of GMP EM if they are inappropriate and to address the specific requirements for GMP EM.

Endnotes

^a<http://www.ec-gis.org/inspire>. ^b<http://portalu.de>.

Authors' contributions

GS performed the GIS and statistical analysis. WS conceived the study, participated in its design and coordination, and drafted the manuscript. Both authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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