Establishment of an Austrian monitoring design to identify potential ecological effects of genetically modified plants

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Abstract

Monitoring changes in species diversity and composition of agro-ecosystems is an important strategy for assessing potential ecological effects of GMP (genetically modified plant) cropping and coexistence. Due to time constraints and limited funding, such monitoring will have to make use of efficient diversity indicators. In the project BINATS (BIodiversity-NATure-Safety) a monitoring network for floristic and faunistic biodiversity of Austrian agro-ecosystems was established. In 2007 and 2008, a first survey of these test sites was undertaken to provide a baseline for the evaluation of possible effects of future GMP cultivation on species numbers and composition.

Introduction

In the European Union in 2008, genetically modified plants (GMP) were cultivated on altogether 107.717 ha in seven member states (Spain, Czech Republic, Romania, Portugal, Germany, Poland and Slovakia, ISAAA 2008). At present, only Bt maize Mon810 is commercialized.

According to the European Directive RL 2001/18/EG, case specific monitoring as well as a general surveillance of potential ecological effects are obligatory measurements accompanying the release of GMPs. They should function as an early warning system for potential negative ecological effects caused by GMPs. **Case specific monitoring** focuses on precise hypotheses referring to the potential effects of the specific GMP on other organisms, on species composition or on specific ecosystem functions on a more local level. Additional ecological parameters such as distance of pollen flow, potential for cross hybridisation, wildness, volunteers as well as agrarian parameters like application of herbicides and altered agricultural practices (Firbank et al. 2003, 2006) have to be investigated in the direct vicinity of the GMP release. The main investigation areas are the agrarian fields themselves and the affected landscape in close proximity to the released GMP.

In contrast, the approach of **general surveillance** focuses on ecological long-term effects of the released GMPs in general. With this strategy it should be possible to detect indirect, unexpected and delayed arising effects also on a larger (regional or national) spatial scale as well as on a long-term scale. Since general surveillance is not focused on a specific GMP and its known or expected specific impacts, a broader approach is needed. Measuring biodiversity and species composition are an adequate approach. Biodiversity includes the floristic and faunistic richness of species but also the diversity of habitats and their cross linking of communities. Since biodiversity as a whole is not measurable in field trials, an adequate selection of biodiversity indicators such as e. g. the use of cross-taxon correlation in diversity patterns (Sauberer et al. 2004) and the selection of organism with particular vulnerability to GMPs is a prerequisite. Using these indicators it should be feasible to get a realistic picture of biodiversity in the agrarian regions and to be able to monitor and evaluate GMP specific effects such as the possibility of hybridisation or abundance changes of sensitive organisms. Moreover, time and financial constrains enforce the use of effective indicators.

For accomplishing the different purposes of the two monitoring approaches, different monitoring designs related to parameters and scale of investigation, etc. will be necessary (Pascher et al. 2007). For both strategies - the case specific monitoring as well as the general surveillance - comparative data of the initial state (without GMPs) are a necessary basis to be able to detect changes due to GMP release (Pascher et al. 2000). Time series data are required which characterise the general trend to be able to isolate specific effects attributed to a GMP release. Furthermore, reference data on areas with no GMP impact are essential for comparison. However, effects on species diversity and composition as well as on related ecosystem functions typically manifest themselves with a time lag, depending on the resilience and resistance of ecosystems. Thus, short-term monitoring might be inadequate for detecting long-term effects. Monitoring systems have to be designed in a way that repetition of the implemented trials is possible in a comparative way, even after many years.

One of the main tasks of a monitoring system is to provide the *status quo* as a benchmark for comparison. It should allocate reference sites and be representative for the area of interest. Over the long run, it should provide a time series of observations to figure out the general trend and it should be designed sustainable with the possibility to re-observe the same set of test sites over a long-term scale. Beside a temporal approach, also a spatial approach is suggestive for a GMP monitoring. In case of a GMP release, cultivation areas with GMPs have to be compared with GMP free areas to be able to detect differences. On a practical view, a GMP survey should be part of a national environmental monitoring system to maximize the synergetic potential.

Protecting biodiversity is of high value to our society. It is a legal regulation as well as the will of the society (Rio Declaration on Environment and Development 1992). Europe’s nature conservation policy focuses on creating a network of sites called Natura 2000, designed to protect the most seriously threatened habitats and species across Europe. The legal basis for Natura 2000 is the Birds’ Directive (79/409/EEC) and the Habitats’ Directive (92/43/EEC). The law additionally enforces that detrimental implications to biodiversity are to be avoided. Presumably, the new agricultural GM technique poses an additional factor for unintended loss of biodiversity in agricultural areas. Influencing factors are the anticipated changes in landscape structures such as further enlargement of fields for GM cropping and as a consequence the removal of field edges and altered agricultural practices as demonstrated in the British Farm Scale Evaluations tracking back to, e. g., time of herbicide application (Firbank et al. 2006). Furthermore, gene transfer from GM crops to wild relatives (e. g. Chevré et al. 2004, Warwick et al. 2008), persistence of feral GM crops outside cultivation (e. g. Crawley & Brown 2004, Pascher et al. 2000, 2006, Pessel et al. 2001, Pivard 2007) and the potential suppression of native plants or other organisms will also exert influence on biodiversity loss. Firbank et al. (2003, 2006) demonstrated that there are adverse effects of genetically modified herbicide-tolerant crops on farmland biodiversity. Decreasing weed biomass and seed production with cascading effects through food chains have already been proven (Bohan et al. 2005).

In connection with GMP cultivation, nature conservation areas will not be the primary target-areas of potential impacts of GMPs but the agrarian region itself will be. As the functional importance of the organisms of an agro-ecosystem focus on the cultivated area itself assessments should not be restricted to marginal or uncultivated habitats only but also consider the cropped area (Büchs 2003). Each European country is required by the European Union to design a national specific monitoring plan which should account for regional and agrarian specialities.

The Austrian alignment and safeguard measures focus on the precautionary principle and also take “uncertainties” into consideration in the risk assessment and the design of a GMP monitoring as laid down in the Directive 2001/18/EC and in the Regulation 178/2002. In Austria, no GMP has ever been released, neither for testing nor for placing on the market. Consequently, there is no direct experience with a practical implementation of a GMP monitoring in Austria. Furthermore, the knowledge and the evaluation of biodiversity in the Austrian agrarian regions are still in a beginning stage (Sauberer et al. 2004, Traxler et al. 2005). For classifying a regional risk of a GMP relevant floristic and faunistic data have to be compiled with a systematic monitoring design.

As prescribed in the directive, each member state's specific initial agrarian situation is the basis for a regional evaluation. Austria - 84.000 km^2 in size - shows a very special agricultural setting which is unique in the European Union. On general, a small scale agriculture is typical for Austria with an average field size of only 1 ha. While parts of the Austrian agricultural region have already been converted to a modern agricultural landscape with larger fields and higher fragmentation of semi-natural and natural habitats, other parts are still present, where a complex and fine grained landscape mosaic with small fields and a high portion of semi-natural habitats is conserved (Wrbka et al. 2008). Austria is the EU member state with the largest portion of organic farming (14%, approx. 20 000 organic farms). This agricultural system requires the most careful consideration in coexistence due to the strived threshold for GM contamination in organic products of only 0,1% (Pascher & Dolezel 2005). Especially the thermophile Pannonian Region of Austria is rich in many thermophilic species. Therefore, this region deserves special significance in connection with coexistence, because some of these additional species function as potential hybridisation partners of GM crops. Biodiversity is often considered exclusively in connection with highly protected areas, but in Austria also man made or influenced areas may possess high diversity.

Aim of the project

Within the four year study BINATS (BIodiversity NATure Safety) we established a long-term monitoring network for assessing biodiversity in respect to possible detrimental effects of GMP release. We identified appropriate and practicable indicators considering factors such as suitability as a biodiversity indicator, cost and time efficiency in a permanent monitoring as well as being indicative for certain GMP specific effects like gene-transfer, vulnerability against changing agricultural practice or being sensitive against specific GMP traits (e. g. Bt). The assembled ecological biodiversity data will be the basis for future investigation of potential effects of the GM cropping (Pascher et al. 2000). Also the regional occurrence and frequency of potential hybridisation of species were surveyed in this study.

Methods

Establishment of a network of monitoring sites and selection of 100 BINATS test sites

First of all, a stratified random sampling was performed (Pascher et al. 2007, 2008). The stratification procedure accounted for both environmental (temperature and precipitation) and land use variables (current land use, regional cultivation of oilseed rape and maize (figure 1), the extent of grassland and forest and the diversity of soil types). Ten groups of strata with different characteristics were calculated. A total of 65 test sites (50 test sites and 15 additional ones to have a sufficient number of test sites for unforeseen failures) were selected corresponding to these strata in both oilseed rape and maize cultivation areas, from a total of 1144 and 1568 potential ones, respectively. Altogether 1360 farmers were instructed about the project BINATS and the details of the planned monitoring in their fields such as the methodological approach, time setting and number of field workers. Considering the negative responses of the Austrian farmers in 22 and five test sites, respectively, test spots or test sites as a whole, had to be repositioned. In these areas land owners refused admittance to their fields. In the finalized selection procedure these objections were considered to definitely fix 50 test sites each for maize and oilseed rape (figure 1). A first power analyses demonstrated that with the chosen sample size used in this baseline survey, species number changes between five and 25% - depending on the indicator taxon - may be detected in future (Pascher et al. 2008).

Setting of the test sites

The size of the test sites was defined with 625 x 625 m. To be consistent with other monitoring programs, the grid of the Austrian Forest Inventory was used. The application of an identical spatial arrangement should help that different Austrian monitoring programs could mutually use these data. One main objective in future focuses on linking the data of different biodiversity programs to accomplish a broader general data basis which could also enlarge the data base to be available for the ecological evaluation of GMPs. Within each test site, ten test circles of 20 m radius were randomly distributed for investigation of species (figure 2).

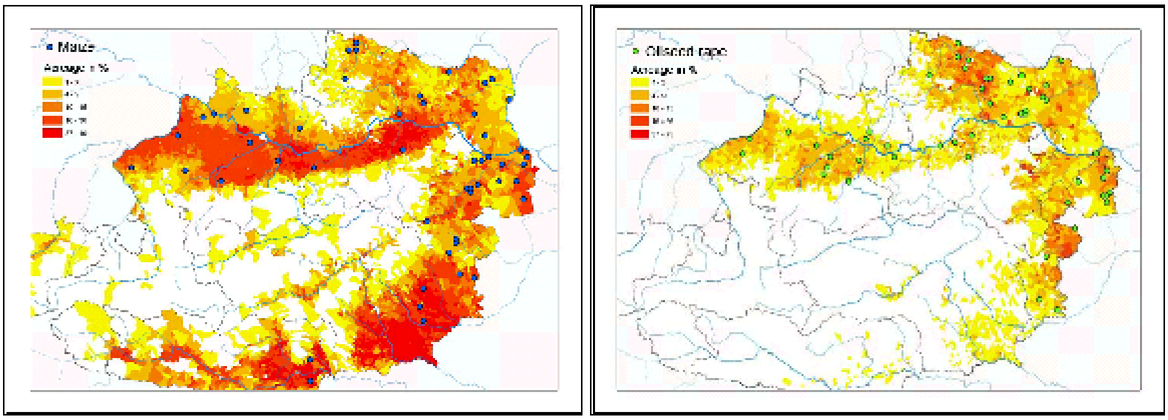

Figure 1: Position of the 50 test sites each, in the maize and oilseed rape cultivation area.

Figure 2: The setting of the BINATS test sites: 625 x 625 m, ten randomly distributed test spots with 20 m radius in the highly structured landscape of Großwetzdorf in the Weinviertel.

Choice of adequate biodiversity indicators in a GMP monitoring

The development and establishment of a GMP monitoring which will attend a future release of GMPs will be faced with limited costs and time. So, the choice of the biodiversity indicators has to comply with representativeness for cross-taxon diversity and cost-efficiency in a permanent monitoring system.

Vascular plants are amongst the best surrogates for total species numbers (Sauberer et al. 2004), they are comparatively easy and fast to record and are sensitive to direct and indirect effects of GVP cultivation.

Butterflies meet the requirements of a general surveillance as well as of a case specific monitoring. Numerous maize pollens are found on fodder plants of the caterpillars of some butterfly species in close proximity to the fields. Although controversially discussed, some studies demonstrated that the consumption of these GM contaminated fodder plants negatively affected their development (e. g. Felke et al. 2002). Moreover, due to its special demands this indicator also predicts the ecological quality of the test site.

Grasshoppers are also well correlated with the species number of other taxonomic groups (Sauberer et al. 2004). In comparison with other insect taxa (e. g. carabide beetles) this indicator can be captured with smaller cost and time effort. The grasshoppers are identified acoustically and optically directly in the field, no positioning of soil entrapments is necessary.

Landscape structures correlate well with diversity of organisms, are easy to record and provide data for the whole test site.

Method of inventory

Landscape elements were mapped for the whole test site using the Austrian Red Data Book of Endangered Biotopes revised and adapted to the GMP monitoring program. Vascular plants, butterflies and grasshoppers were recorded within a test spot with a radius of 20 m (figure 2). A cross transect (north, south, west, east, 4 x 20 m x 2 m) through each spot served as the basic sampling area for these taxa (figure 3).

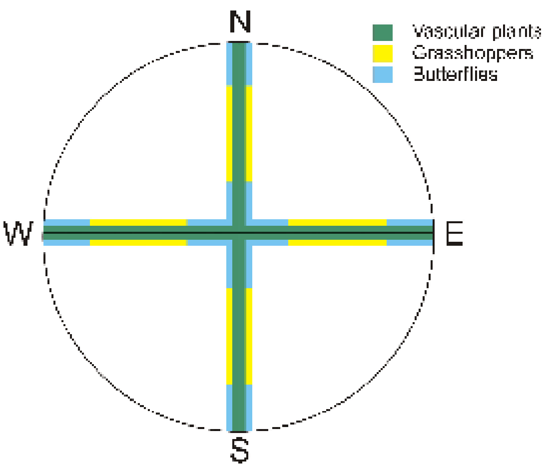

Figure 3: Inventory design of the cross transect.

Vascular plants were sampled twice a year - in spring and in summer - and recorded separately within each habitat type along the cross transect. The abundance of relevant hybridisation partners of oilseed rape was estimated on an ordinal scale.

Butterfly species were sampled once in each transect (20 m in length, 5 m in width) between mid July and mid August taking account of a temperature threshold of maximal 36°C. At these temperatures, butterflies intensely reduce their activity.

Grasshopper species and abundance were surveyed on the same cross transect (10 m in length, starting 5 m from the centre of the spot, 5 m in width) within acoustic and optic observation units of eight minutes.

Results

In several Austrian agricultural regions, biodiversity turned out to be rich concerning habitats as well as species in comparison to other European countries with large scale agriculture e. g. the Netherlands. In all 100 investigated test sites altogether exactly 900 vascular plant species were found. For comparison, there are altogether 2.950 vascular plant species in Austria including the unique alpine flora. That means a little bit less than one third of the entire Austrian flora was found in the investigated agricultural regions. With 519 detections in different habitat types during both inventories in spring and summer, Creeping Thistle (*Cirsium arvense*) proved to be the most prevalent species followed by Common Chickweed (*Stellaria media,* 471) and White Goosefoot (*Chenopodium album* agg., 419). Fifty-three different species of grasshoppers with the prevalent species *Chorthippus biguttulus* (427 detections in different transects) and 41 butterfly species - with the Small White (*Pieris rapae,* 204) being dominant - were registered in altogether 1000 cross-transects. The species number of vascular plants within a single test site – pooled from observations of all ten cross transects within each test site - varied between 214 as a maximum and 22 as a minimum, grasshoppers between 20 and six and butterflies showed a maximum of 15 species. In 58.3% of the test spots no butterfly could be observed.

Several recorded species are listed in the Red List of Endangered Species in Austria, vascular plants such as *Adonis vernalis*, *Pulsatilla pratensis* subsp. *nigricans*, *Papaver argemone*, *Sagina apetala*, *Ranunculus arvensis* and *Centaurea cyanus* to mention some of these. Other species are strongly protected by law in some Austrian provinces e. g. *Agrostemma githago* or *Linaria arvensis*. Twenty-one of the 43 registered grasshopper species are endangered to different degrees. Dusky Large Blue (*Maculinea nausithous*) and Chalkhill Blue (*Polyommatus coridon*) are examples of two detected endangered butterfly species. These results highlight the value of Austrian agricultural areas for biodiversity.

Looking at the structure of agricultural landscapes, a maximum number of 36 different biotope types could be mapped in a single test site, as a minimum only four. The maximum of polygons in a highly structured test site was 315 (figure 2).

In Austria more than twenty taxonomically closely related species of the tribus Brassiceae occur (Pascher et al. 2000). Because of their close relationship to oilseed rape they could function as hybridisation partners (e. g. Chevré et al. 2004). A comparison of maps shows that the main distribution area of the related species correlates with the areas where oilseed rape is cultivated in Austria.

Consequently, another component of the monitoring was to establish the occurrence and frequency of Brassicacean species within the ten test spots of each investigated test site to get a first clue of the likelihood of hybridisation events. Additionally, the habitats in which these plants occurred were mapped. In almost three quarters of the test sites – 71 out of 100 – oilseed rape occurring as feral population or volunteer was registered. On average, feral or volunteer oilseed rape was detected on 2.41 test spots in the 71 test sites with a majority of volunteer plants in adjacent cultures. In 8% of the test sites, feral oilseed rape was found along road verges tracking back to seed spillage events during transportation. In consequence, transport loss is a major factor for the establishment and persistence of feral oilseed rape populations outside cultivation (Pivard et al. 2007). These populations have the potential to serve as sources, corridors and stepping stones for gene transfer (Pessel et al. 2001). The origin and population dynamics of feral populations of oilseed rape have already been studied in different parts of Europe (France: Pessel et al. 2001, Pivard et al. 2007; Great Britain: Crawley & Brown 2004). In Austria most tested feral populations proved to be persistent (Pascher et al. 2000, 2006). The varied study results suggest that the origin and persistence of feral oilseed rape populations cannot be harmonized in different parts of Europe. Hence, a regional case specific evaluation of feral plants is crucial and has to be part of a future monitoring program of GMPs. In Austria feral oilseed rape populations are becoming increasingly frequent along roads, country roads, motor ways and railways. Their blossoming is not limited to special periods in spring but the feral plants flower and produce seeds from March to November (Pascher et al. 2000).

Discussion

With the project BINATS a long-term monitoring network for floristic and faunistic biodiversity of the agricultural regions in Austria was established. The sampling design guarantees representativeness for all of Austria. The project provides the first comprehensive data of the diversity of landscape structures and of three different organism groups across the agricultural regions throughout Austria (Pascher et al. 2008). In case of a GMP release comparative test sites and their biodiversity data will immediately be available. Furthermore, a first case specific as well as a general assessment of regional potential risk is already possible on the basis of these data. The efforts which such an ecological monitoring program of GMPs will entail can now be calculated in detail in costs and time (Pascher in prep.).

The four surveyed indicators give a comprehensive picture of biodiversity in the Austrian farmland. A common monitoring design was developed taking the special investigation of each indicator into account. Because of cost limits only one butterfly inventory could be implemented which proved to provide only fragmentary data – in less than half of the investigated cross transects butterfly species could be recorded. The statistic calculation of Bühler (2007) suggests that five to seven repetitions of inventory would be necessary to capture almost all butterfly species in the agrarian regions. To serve as a significant indicator, butterfly monitoring needs supplemental funding or it has to be abandoned.

Existing Austrian monitoring programs will not adequately meet all the demands of a general surveillance program of GMPs. The BINATS design is a cost-efficient general means for monitoring the biodiversity effects of different agricultural schemes and their eventual large scale coexistence. The program provides a methodical and organisational framework into which additional faunistic indicators - if needed – may easily be integrated in the future.

The monitoring data serve as a baseline (*status quo*) to observe possible effects in an ecological risk assessment of potential future releases of genetically modified crops in Austria. They can be used as basic data for case specific investigations (evaluation of the specific crop, the trait, regional aspects, etc.) as well as for general surveillance. Once, the monitoring is running and we will know more about the general trends it should be possible to isolate GMP specific effects on biodiversity or landscape structure.

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