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# Case-specific monitoring of butterflies to determine potential effects of transgenic *Bt*-maize in Switzerland

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ABSTRACT

The present study investigated whether case-specific monitoring (CSM) would be an appropriate option to detect possible effects on butterflies during commercial cultivation of *Bt*-maize (Cry1Ab). The analysis of an existing dataset on butterfly communities in Switzerland allowed quantifying the variability in species richness and abundance of nine ecological groups of butterflies resulting from habitat features, agricultural field management, landscape features, regional farming characteristics, spatial variability and temporal fluctuations. The data set also enabled to estimate the sample size needed to detect potential effects of *Bt*-maize in a CSM programme within the determined variability. Fifteen of the 24 tested descriptors of environmental and farming context induced significant variability in butterfly species richness and abundance of generalist butterflies. In case regulatory authorities would request a CSM, a stratified sampling design considering habitat, landscape, and regional farming types would be essential to account for existing sources of variability. However, recording only the presence and abundance of butterfly species will not be sufficient to explain occurring changes and sampling of additional explaining variables is crucial. The analysis showed that CSM will at best detect large effects on ubiquitous butterflies, whilst the detection of small effects would need a considerable sampling effort. A sampling effort of 100 pairs of fields or field margins will only allow detecting changes exceeding 30% in species richness or abundance of the most abundant species. Rare butterfly species can hardly be monitored in CSM and causalities between changes in butterfly communities and the cultivation of *Bt*-maize will be difficult to determine due to the high variability of communities and the multitude of influencing environmental factors. CSM is thus unlikely to reduce remaining uncertainties on potential effects on butterflies during commercial cultivation of Bt-maize. Ultimately, potential effects might be evaluated more rigorously in pre-market risk assessment by studying hazard and exposure of sensitive butterfly stages to *Bt*-maize through experimental toxicity studies in the laboratory or in the greenhouse.

## 1. Introduction

In the initial risk assessment prior to the registration of *Bt*-maize expressing the insecticidal protein Cry1Ab from *Bacillus thuringiensis* in the United States, the U.S. Environmental Protection Agency (EPA) concluded that the impact of pollen of *Bt*-maize on non-target butterflies would be minimal because of their low exposure to the toxin (EPA, 2001). Later on, the case of the monarch butterfly (*Danaus plexippus*) caused much public interest and led to a debate over potential risks of *Bt*-maize on non-target butterflies (Berenbaum, 2001). In a number of extensive studies, it could be shown that *Bt*-maize poses a negligible risk to monarch butterfly populations due to the low amount of toxin contained in the pollen and due to low larval exposure to *Bt*-pollen (Sears et al., 2001; Dively et al., 2004).

A number of studies have been published assessing potential risks of Bt-maize expressing Cry1Ab on European butterflies. In a theoretical exposure assessment, Schmitz et al. (2003) estimated that approximately 7% of the German butterfly species could potentially be affected by *Bt*-pollen exposure at a national scale as they occur in farmland areas. On a regional scale, however, only 14% of these (=1% of the total) were found to be potentially exposed. In an experimental exposure assessment, Gathmann et al. (2006a) studied the distribution of larvae of the small tortoiseshell (*Aglais urticae*) and its host plant, the stinging nettle (*Urtica dioica*). In field margins of maize fields, only a few nettle stands and no larvae were found making it questionable whether *A. urticae* is significantly exposed to maize pollen on a population level.

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Toxicity of pollen from *Bt*-maize event Bt176 (that contains high toxin levels but has meanwhile been withdrawn from the market) was shown for larvae of five butterfly species (Felke et al., 2002; Felke and Langenbruch, 2003; Lang and Vojtech, 2006). Interestingly, three of the assessed species (*Pieris brassicae*, *Pieris rapae* and *Plutella xylostella*) are usually regarded as pest species that are controlled in other crops. Only one study assessed the impact of the currently commercialized *Bt*-maize event MON810 on butterfly larvae and found no differences in their abundance on artificially sown weed strips near *Bt*- and non-transgenic maize fields (Gathmann et al., 2006b).

The available data provide confidence that the commercial cultivation of the currently available *Bt*-maize varieties comprises a negligible risk for European butterfly species. This conclusion is based on the assumption that larval exposure to Cry1Ab is relatively low for European butterfly species considering its low expression level in pollen of the only approved *Bt*-maize event MON810, the fact that most pollen is deposited within a few meters from the field border (Pleasants et al., 2001; Dupont et al., 2006), and that maize is not considered a host plant for non-target butterflies. Given the effort involved it is impossible to test the toxicity of Cry1Ab on all potentially exposed lepidopteran species, in particular those endangered or threatened, prior to commercial approval in pre-market risk assessment (PMRA). One way to cope with the remaining scientific uncertainties inherent to risk analysis and to the scientific process is post-market monitoring (PMM) as mandated by EU legislation (European Community, 2001). In addition to PMRA, notifiers (i.e., usually the company marketing a GM crop) must submit a PMM plan for each transformation event to ensure the detection of adverse environmental effects possibly deriving from its commercial cultivation. According to EU legislation, PMM is divided into case-specific monitoring (CSM) and general surveillance (GS) (European Community, 2001; European Council, 2002). CSM is focussing on anticipated effects of a specific GM crop on the environment and aims to assess whether these effects do occur during commercial cultivation. It is not required where the conclusions of PMRA identify an absence of risk or negligible risk (European Council, 2002), but must be initiated if remaining uncertainties arise from PMRA and justify further inquiry. General surveillance, in contrast, aims at detecting adverse effects on the environment that were not anticipated during PMRA and has to be performed in any case.

Several conceptual proposals have been made how PMM programmes could be designed to yield the requested results (e.g., ACRE, 2004; Sanvido et al., 2005; EFSA, 2006). An important point includes the premise that CSM would only have to be performed in case PMRA has resulted in substantial scientific uncertainties that are sustained by a plausible risk hypothesis (Sanvido et al., 2005; EFSA, 2006). Although the risk assessment of current *Bt*-maize varieties shows no indication for a particular risk, it has been proposed to include non-target butterflies in a respective PMM programme (Schmitz et al., 2003; Graef et al., 2005; Gathmann et al., 2006a). Given the specific hypothesis that Cry1Ab could harm non-target butterflies, a PMM of potential effects of *Bt*-maize on butterflies would typically require a CSM-approach. Two important distinctions to other programmes have to be made in case a CSM-approach would have to be implemented. First, CSM should include all species that could potentially be exposed to *Bt*-maize. This is in contrast to PMRA studies, which have usually concentrated on one or a few endangered or threatened butterfly species of particular interest (Sears et al., 2001; Peterson et al., 2006). The findings obtained from testing or assessing a limited number of butterfly species were then used to draw more general conclusions on the risk for a majority of species. Second, CSM should provide scientific data for later decision-making processes. This requires designing CSM protocols that determine, as unambiguously as possible, the reasons for potential declines in butterfly populations.

The aim of the present study was to investigate both the effort involved and the challenges when developing a CSM protocol for *Bt*-maize (Cry1Ab) that should allow detecting potential adverse effects on butterflies. An important factor consisted in using a hierarchical approach to address issues of environmental variability at the field, landscape and regional scales. For the first time, the design of a CSM protocol was based on an existing extensive dataset on butterflies in Swiss agricultural landscapes (Aviron et al., 2009) that enabled to characterise the variability of butterfly communities linked to environmental conditions and farming activities. The specific objectives of the study were: (1) to identify butterfly species, which might be exposed to *Bt*-maize pollen in Switzerland, (2) to identify existing variability of butterfly communities due to environmental conditions and farming activities, (3) to estimate the effect size (i.e., the magnitude of *Bt*-effects that could be detected) based on existing variability in butterfly communities, and finally (4) to estimate the statistical power and corresponding sample size needed to detect significant effects of *Bt*-maize on butterfly communities in CSM, whilst accounting for the existing variability.

## 2. Materials and methods

### 2.1. Used dataset and tested environmental factors

The analyses performed in the present study were based on a dataset from a monitoring project on ecological compensation areas and biodiversity in Switzerland (Aviron et al., 2009). In three agricultural regions (7–8 $km^2$ each) representative of current farming practices in the Swiss lowlands (arable, mixed arable-grassland, grassland), butterflies were observed every 2 years from 1998 to 2004 in several habitat types including low-input and conventional meadows, hedgerows, orchards, flower strips and crop fields. In addition, an extensive dataset containing 24 variables describing the environmental context and agricultural management of sampled habitats was recorded. Because agricultural management of sampled habitats was only recorded in 1998, 2000 and 2002, the analyses of butterfly observations in the present study were restricted to the first 3 sampling years. This led to a sub-sample of 358 sampling sites over the 3 years: 129 low-input meadows, 79 conventional meadows, 33 hedgerows, 41 orchards, 22 flower strips and 79 crop fields. Apart from hedgerows and orchards, only a few sites could be observed throughout the entire sampling period because crop rotations lead to a very dynamic land-use in agricultural landscapes.

Species and individuals of adult butterflies were counted during five 10 min. observation periods between May and August, across an area of 0.25 ha in the middle of fields or along hedges. The data collected during the five periods per site were pooled for the analysis. From 1998 to 2002, a total of 41 butterfly species with 19,316 individuals were recorded. In addition, botanical diversity was assessed at each sampling site over an area of 100 $m^2$ according to the Braun–Blanquet method. Since botanical diversity was highly correlated and redundant with habitat types, the obtained data was not integrated in the final analyses.

Data on the environmental and agricultural context of sampling sites included 24 variables describing habitat type, local site conditions, agricultural management, landscape context of sampled habitats, spatial variability, and regional farming characteristics (Supplementary Table 1). Habitat types were grouped into six categories (low-input meadow, conventional meadow, hedgerow, orchard, flower strip, crop field). Local site conditions were described by field orientation and slope. Data on field management, namely farm context (farm size; production type; %