An approach for post-market monitoring of potential environmental effects of *Bt*-maize expressing Cry1Ab on natural enemies

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biological control, case-specific monitoring, environmental monitoring, general surveillance, non-target risk assessment, transgenic crops

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Abstract

Post-market monitoring (PMM) consistent with Swiss and European Union legislation should ensure the detection and prevention of adverse effects on the environment possibly deriving from commercial cultivation of genetically modified (GM) crops. Insect-resistant GM crops (such as *Bt*-maize) raise particular questions regarding disturbances of biological control functions provided by beneficial insects such as predators and parasitoids (so-called natural enemies). Consensus among regulators, scientists and the agricultural biotech industry on appropriate PMM plans allowing the detection and possibly prevention of such adverse effects is still lacking. The aims of this study were to identify the necessity for PMM of *Bt*-maize expressing Cry1Ab on natural enemies and to develop an appropriate PMM plan. The approach chosen consisted in determining what type of monitoring is most appropriate to address potential effects of *Bt*-maize on natural enemies during commercial cultivation. This included identifying whether there remain substantial scientific uncertainties that would support case-specific monitoring. Existing pre-market risk assessment data indicate that *Bt*-maize (Cry1Ab) comprises a negligible risk for disturbances in biological control functions of natural enemies. As a consequence, a faunistic monitoring of specific groups of natural enemies is not considered an appropriate approach to detect failures in biological control functions. Alternatively, an approach is proposed that consists in indirectly analysing biological control functions by surveying outbreaks of maize herbivores. Unusual herbivore outbreaks could indicate failures in biological control functions of natural enemies. Data could be collected via questionnaires addressed to farmers growing *Bt*-maize. Significant correlations between unusual occurrences of specific maize herbivores and the cultivation of *Bt*-maize would subsequently need specific studies to determine possible causalities in more detail. The here proposed approach has the advantage of covering different natural enemy groups. It represents a cost-effective strategy to obtain scientifically sound data as a basis for regulatory decision-making.

Introduction

Both Swiss and European Union (EU) legislation aim at ensuring a high level of protection of the environment. This includes the management of potential risks resulting from the deliberate release of genetically modified (GM) organisms into the environment (GTG, SR 814.91; European Community

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2001; European Union 2003). Approval for commercial cultivation of a specific transformation event is based on a pre-market risk assessment (PMRA) where potential adverse effects of the GM plant on the environment are assessed on a case-by-case basis. Approval is only granted if the risk assessment indicates that the risk of the GM crop on the environment is sufficiently small to be acceptable. Risk assessments are, however, not absolutely free of uncertainties (Hill and Sendashonga 2003; Levidow 2003; Sanvido et al. 2005). As a way to cope with the scientific uncertainties inherent to risk analysis and to the scientific process, notifiers (i.e. usually the company marketing a GM crop) must submit a post-market monitoring (PMM) plan when requesting approval for commercial cultivation of a specific GM plant. PMM plans should ensure the detection and prevention of adverse effects on the environment possibly deriving from commercial cultivation of GM crops. 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 adverse effects of a specific GM crop and aims to assess whether these effects on the environment occur during commercial cultivation. The decision to initiate a CSM programme necessitates sufficient remaining scientific uncertainties arising from PMRA that would justify further inquiry, for example, the aim to manage potential risks that could not be adequately addressed because supposed effects may only appear after large-scale releases. GS, in contrast, aims at detecting adverse effects on the environment that were not anticipated during PMRA. Different necessities regarding the two types of monitoring programmes are specified in the legislation. While GS has to be performed in any case, CSM may not be required when the conclusions of PMRA identify an absence of risk or negligible risk (European Council 2002). Ultimately, the European Commission and the Member States define the PMM activities needed when issuing consent for commercial approval of a specific GM crop. After implementation of the PMM plans, both the competent national authorities and the European Commission collect the data to decide on maintaining, renewing or withdrawing commercial approval of a specific GM crop.

Several conceptual proposals have been made how PMM programmes could be designed (e.g. Wilhelm et al. 2003; ACRE 2004; Graef et al. 2005; EFSA 2006; Jepson 2006; Tinland et al. 2007). We have developed a framework proposing structures and procedures for PMM of GM crops (Sanvido et al. 2005). For CSM a bottom-up approach was proposed, which includes the identification of a risk hypothesis to determine whether a specific GM crop could cause a relevant harm to a particular environmental resource. The risk hypothesis should thereby be both plausible and testable to be confirmed or rejected after a defined period of time. For GS, in contrast, a top-down approach was proposed, suggesting to concentrate on the subjects of environmental concern (so-called protection goals) that need to be preserved and that should not be adversely affected by GM crop cultivation, or by any other factor in general. Unanticipated environmental damage in the defined protection goals may be detected by using existing monitoring networks and by establishing appropriate reporting systems to identify adverse incidents. Any result from GS would, however, not be directly linked to any specific attribute of GM crop cultivation; therefore causality between detected damages and the cultivation of GM crops would have to be determined via additional, specifically designed, studies. The decision to investigate such a causality would have to be based on the plausibility that the cultivation of a specific GM crop could have caused the detected damage (Sanvido et al. 2006).

A need to define the conditions and measures required for PMM on a scientific, legal, and administrative basis has become apparent as the EU entered the first GM maize varieties expressing the insecticidal protein Cry1Ab from *Bacillus thuringiensis* into the Common EU Catalogue of Varieties in 2004. *Bt*-maize expressing Cry1Ab was initially developed to control larvae of the lepidopteran pest European Corn Borer (*Ostrinia nubilalis*; Lepidoptera: Crambidae), but has also shown to be effective against larvae of the Mediterranean corn borer (*Sesamia nonagrioides*; Lepidoptera: Noctuidae) (Gonzales-Nunez et al. 2000). The cultivation area of *Bt*-maize in the EU has gradually increased over the past years, especially in areas where the two pests cause serious infestations. In 2007, the *Bt*-maize area grown in the EU exceeded 110 000 hectares with the highest share being grown in Spain (75 000 ha), followed by France (22 000 ha), the Czech Republic (5000 ha), Portugal (4300 ha) and Germany (2700 ha) (James 2007). The cultivation of insect-resistant GM crops such as *Bt*-maize raises particular questions regarding potential harm to organisms other than the pest(s) targeted by the expressed toxin. One hypothesis is that *Bt*-maize could alter biological control functions of beneficial insects such as predators and parasitoids (so-called natural enemies), which are important for controlling herbivorous insect populations in the crop (Romeis et al. 2008b). The potential for adverse effects of *Bt*-maize on natural enemies has been evaluated as part of PMRA prior to the decision to cultivate these crops commercially. Albeit no significant risk to the environment was found in these evaluations (EPA 2001; Mendelsohn et al. 2003), the necessity, extent and design of appropriate PMM plans to detect potential adverse effects of *Bt*-maize on natural enemies are discussed controversially among different EU regulatory bodies, scientists and the agricultural biotech industry. Although conceptual differences between CSM and GS have been identified (ACRE 2004; Sanvido et al. 2005, 2006; EFSA 2006), the distinction between the two programmes still remains unclear among many participants involved in the discussion. Particular ambiguity remains on the question how far results obtained in PMRA have to be confirmed by CSM and what conditions are necessary to choose either a CSM- or a GS-approach when addressing a specific question. Some have proposed to elaborate checklists for individual groups of GM crops and for different transgenic traits that would lead to specific sets of monitoring parameters, criteria and methods (Züghart et al. 2008). Given that these checklists relate to potential anticipated effects of GM crops, it seems that they are intended to be used in CSM to confirm the results obtained in PMRA on a larger scale. For insect-resistant *Bt*-maize, for example, this would include monitoring potential effects on herbivores and their natural enemies (Züghart et al. 2008), independent from the probability that a particular effect may occur. In contrast, a more pragmatic approach has been proposed, that is, to perform CSM only in case PMRA would have resulted in sufficient scientific uncertainties that would be sustained by a plausible risk hypothesis (Sanvido et al. 2005; EFSA 2006). This is based on the rationale that CSM is not a programme to confirm principally the absence of effects on a commercial scale, but an option to cover remaining uncertainties and to manage an identified risk (such as insect resistance management measures for *Bt*-maize to delay potential resistance development in the lepidopteran target pests). Similar to environmental risk assessment (Raybould 2006, 2007; Romeis et al. 2008a), a consistent problem formulation is ultimately a prerequisite to determine the need for and the scope of CSM activities. PMRA results should not be confirmed by CSM as a matter of principle, but only if, for example, there is evidence for a scale-dependency of potential effects. Without a clear hypothesis regarding a potential adverse effect at the commercial scale, there is no rationale for CSM.

Given that a comparable regulation is not required for conventional plants, the adoption of the new legal requirements related to the commercial cultivation of *Bt*-maize represents a challenge for scientists, the agricultural biotech industry and the different EU regulatory bodies. As yet, there is no consensus on how PMM plans of *Bt*-maize could be implemented in practice to yield data that can be used for regulatory decision-making. This lack of consensus inevitably leads to a certain confusion regarding decisions on the implementation of appropriate risk management measures. The aim of this study was to develop a PMM plan for insect-resistant *Bt*-maize expressing Cry1Ab-proteins that allowed detecting potential adverse effects on natural enemies during commercial cultivation. The specific goals of the study were (i) to identify the necessity for PMM of *Bt*-maize on natural enemies and (ii) to develop a standard plan for PMM of *Bt*-maize on natural enemies that addresses the identified needs.

Approach

Consistency with our previously proposed risk assessment (Dutton et al. 2003; Romeis et al. 2008a) and monitoring (Sanvido et al. 2005, 2006) frameworks is an important aspect of the PMM plan we describe here for insect-resistant *Bt*-maize and natural enemies. The knowledge gained during PMRA should be taken into account when identifying the need for PMM of *Bt*-maize on natural enemies (Sanvido et al. 2005; EFSA 2006). It is important to recognize that Cry1Ab expressing *Bt*-maize varieties subject to PMM have passed a regulatory approval process, that is, potential adverse effects have been thoroughly investigated prior to commercial approval in PMRA studies conducted both under contained conditions and, if evidenced, in experimental field studies. Consequently, considerable scientific knowledge is available on non-target effects of *Bt*-maize (EPA 2001; Mendelsohn et al. 2003; Romeis et al. 2006; Wolfenbarger et al. 2008). Considering this knowledge, the approach presented here consisted in determining what type of monitoring (i.e. either CSM or GS) would be most appropriate to identify potential effects of *Bt*-maize on natural enemies during commercial cultivation. It was analysed whether existing PMRA data leave enough uncertainties that

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would justify the performance of CSM activities. This was performed based on a characterization of the risk *Bt*-maize expressing Cry1Ab could pose for natural enemies. The risk characterization of potential effects on natural enemies included both an assessment of the hazard (i.e. the toxicity of the insecticidal protein to natural enemies) as well as an assessment of their exposure level to the toxin in the field.

Identification of the Need for Case-Specific Monitoring

Characterization of the risk of *Bt*-maize on natural enemies

Hazard characterization – mode of action of the Bt-toxin Cry1Ab

The different strains of *B. thuringiensis* contain varying combinations of Cry-proteins (*Bt*-toxins). Cry-proteins specifically bind to receptors in the insect midgut causing the formation of lytic pores in the epithelial cell membrane leading to the death of the insect (Höfte and Whiteley 1989; Schnepf et al. 1998; de Maagd et al. 2001). Individual Cry-proteins have a defined spectrum of insecticidal activity, usually restricted to one particular order or family of insects. Direct toxic effects are caused by biologically active compounds and do only occur if a specific mode of action of the toxin is taking place in the organism. A direct toxic effect of the insecticidal *Bt*-toxin on natural enemies would thus only occur if Cry1Ab would specifically bind to receptors in their midgut. Receptor binding studies have shown that this protein is exclusively active against Lepidoptera (moths and butterflies) (de Maagd et al. 2001). This has been confirmed by environmental risk assessments for the Cry-proteins expressed in *Bt*-maize that have been conducted prior to the registration of the first *Bt*-plants in the United States in 1995 (EPA 2001; Mendelsohn et al. 2003). The results of laboratory tests assessing potential effects on a wide variety of non-target organisms that might be exposed to *Bt*-proteins, amongst others also different beneficial insects, showed that direct feeding of purified Cry1Ab-proteins was not toxic to any of the evaluated beneficial insects. In addition, studies in the laboratory, glasshouse and field confirm that, except for the lepidopteran species the toxin is intended for, Cry1Ab does not appear to cause direct toxic effects on any of the predator and parasitoid groups examined (for review see Romeis et al. 2006).

Exposure assessment of key natural enemies

In order to be affected, natural enemies would have to ingest the insecticidal protein. Ingestion can mainly occur via three ways of exposures. Predators can be exposed directly by feeding on plant material (e.g. leaves, pollen), indirectly by feeding on insects that have previously fed on GM crops (and therefore contain the toxin), or by feeding on honeydew excreted from sap-sucking species (Romeis et al. 2008a,b). For *Bt*-maize events expressing Cry1Ab, the last route of exposure is irrelevant because Cry1Ab is not ingested or excreted by aphids (Head et al. 2001; Raps et al. 2001; Dutton et al. 2002). For the two other types of exposure, most predators commonly occurring in maize in Western and Central Europe are exposed to Cry1Ab in one of their life stages when considering the expression of the insecticidal protein in different plant tissues, the feeding behaviour of both herbivores and predators, and the availability of prey. Commonly grown Cry1Ab maize events Bt11 and MON810 produce very small amounts of toxin in the pollen (<1/100 that of leaves) contrasting GM maize varieties based on Event Bt176 (which were withdrawn from the market in the EU in 2005) that produced high levels of Cry protein in leaves and pollen (Romeis et al. 2008b). Many parasitoid species, in contrast, are not exposed to Cry1Ab, given that adults feed on either honeydew or nectar, which both do not contain the toxin (Romeis et al. 2008b). A few parasitoid species may be exposed as they exploit hosts that have ingested the toxin (Chen et al. 2008; Romeis et al. 2008b).

Risk conclusion

The risk assessment illustrates that the commercial cultivation of *Bt*-maize (Cry1Ab) poses a negligible risk for natural enemies controlling maize herbivores. Although most natural enemies occurring in maize in Western and Central Europe are exposed to Cry1Ab in the field, there is no risk for natural enemies given its specific toxicity to Lepidoptera. This is confirmed by large-scale field studies, which have only revealed subtle shifts in the arthropod community that were caused by the effective control of the target pest (Romeis et al. 2008c; Wolfenbarger et al. 2008). Secondary trophic effects may be caused by changes in the availability and/or the quality of target herbivores with specialist natural enemies depending entirely on the target species. The occurrence of these secondary effects is, however, not restricted to GM technology. Any effective pest-control measure will cause a reduction in the number of target prey and host items, which could consequently affect population densities of related natural enemies. Such effects are generally not considered to comprise a particular risk of insecticidal GM crops (OECD 1993; Romeis et al. 2008c).

In conclusion, as the Cry1Ab proteins expressed in *Bt*-maize lack toxicity to natural enemies, it is impossible to formulate a logical hypothesis on an anticipated effect of *Bt*-maize on natural enemies, which would be necessary for CSM (Sanvido et al. 2005; EFSA 2006). CSM of potential effects of *Bt*-maize on natural enemies is thus not evidenced.

Development of a General Surveillance Programme for Natural Enemies

Given that functional biodiversity of natural enemies can be defined as a general protection goal that should not be harmed, a GS-approach is more appropriate to determine whether functional biodiversity is affected by the cultivation of *Bt*-maize. To develop a GS programme to survey potential unanticipated effects on natural enemies possibly occurring from commercial cultivation of *Bt*-maize, a procedure including six steps is proposed (table 1).

Identification of protection goals

Biological control functions in maize

The development of the GS programme begins by identifying the environmental protection goals to be preserved (table 1). Protection goals are set by public policy and represent environmental entities that are commonly accepted as being valuable for the society and thus need to be protected (Suter 2000; Raybould 2007). Human society obtains a wide array of important benefits from biodiversity and associated ecosystems. Ecosystem services are essential to human existence and operate on such a large scale, and in such complex ways, that most services could not be replaced by technology (Daily 1999). Natural enemies, for example, fulfil relevant ecological functions, given that they contribute to the natural regulation of arthropod pest populations within crop fields in agricultural landscapes (Kruess and Tscharntke 1994; Wilby and Thomas 2002; Kremen 2005; Tscharntke et al. 2005a). As the legislative terms laid down in public policy (such as 'environment' or 'biodiversity') are too vague to be scientifically assessed, specific targets for protection (so-called assessment endpoints) have to be defined (Suter 2000; Raybould 2007). Assessment endpoints are operationally defined by an ecological entity and its attributes (Suter 2000). For this study, natural enemies (predators and parasitoids) were identified as the entity to be preserved and the attribute was defined as the biological control functions performed by natural enemies.

Commonly occurring herbivores and key natural enemy groups in maize

The natural enemy groups ensuring natural pest regulation in maize that could be affected by the commercial cultivation of *Bt*-maize were identified based on the herbivores occurring in maize in Central and Western Europe and on the importance of these herbivores as maize pests (table 2) (Dutton et al. 2003; Scholte and Dicke 2005; Häni et al. 2006). The most

Table 1 Sequential steps for the development of a general surveillance plan to survey potential unanticipated effects of *Bt*-maize on natural enemies (adapted from Sanvido et al. 2006)

	Step	Description
1	Identification of protection goals	Which protection goals should not be affected by the cultivation of GM crops?
2	Definition of environmental quality	What environmental quality should be preserved in the previously defined protection goals?
3	Information collection	Collect reports on disturbed biological control functions via existing surveillance programs and reporting system (e.g. farmer questionnaires)
4	Information analysis and evaluation	Detect changes that lie outside of expected variability. Decide if relevant changes require further investigation
5	Determination of causality	Decide if causality to the cultivation of a specific GM crop is plausible; if yes determine causality through risk assessment studies. Decide whether cultivation of GM crop must be temporarily suspended during investigation, or whether risk mitigation measures are necessary
6	Decision-making	Confirm or reject hypotheses of causality between GM crop cultivation and detected damage based on results of risk assessment studies. Decide whether consent for cultivation of a specific GM crop has to be withdrawn, risk mitigation measures are necessary, or no further action is required

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Table 2 Non-target herbivores occurring on *Bt*-maize expressing Cry1Ab in Central and Western Europe plus natural enemies involved in their regulation (adapted from Dutton et al. 2003; Scholte and Dicke 2005; Häni et al. 2006)

Herbivore	Relevance as maize pest	Predators	Parasitoids
Wireworm *Agriotes* spp. (Coleoptera: Elateridae)	Considered a widespread maize pest, occasionally causes damage	Carabid beetles (Coleoptera: Carabidae) Spiders (Arachnida: Araneae)	
Frit fly *Oscinella frit* (Diptera: Chloropidae)	Considered a widespread maize pest, occasionally causes damage	Lacewings (Neuroptera: Chrysopidae) Carabid beetles (Coleoptera: Carabidae) Spiders (Arachnida: Araneae)	Parasitic wasps (Hymenoptera: Pteromalidae); (Hym: Figitidae)
Black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae)	Considered a maize pest with regional occurrence, occasionally causes damage		Parasitic wasps (Hymenoptera: Trichogrammatidae), e.g. *Trichogramma* spp.; (Hym: Ichneumonidae); (Hym: Braconidae) Tachinid flies (Diptera: Tachinidae)
Aphids *Sitobion avenae* *Metopolophium dirhodum* *Rhopalosiphum maidis/R. padi* (Hemiptera: Aphididae)	Considered a potential maize pest	Lacewings (Neuroptera: Chrysopidae) Carabid beetles (Coleoptera: Carabidae) Rove beetles (Coleoptera: Staphylinidae) Ladybirds (Coleoptera: Coccinellidae) Flower bugs (Heteroptera: Anthocoridae) Hoverflies (Diptera: Syrphidae) Midges (Diptera: Cecidomyiidae)	Aphid parasitoids (Hymenoptera: Aphidiidae)
Thrips *Frankliniella* spp. (Thysanoptera: Thripidae)	Considered a potential maize pest	Lacewings (Neuroptera: Chrysopidae) Flower bugs (Heteroptera: Anthocoridae)	
Spider mites *Tetranychus urticae* (Acarina: Tetranychidae)	Considered a potential maize pest	Ladybirds (Coleoptera: Coccinellidae) e.g., *Stethorus* spp. Predatory mites (Acarina: Phytoseiidae)	

important and most regularly occurring maize pest in Central and Western Europe is the European corn borer (*Ostrinia nubilalis*, Lepidoptera: Crambidae). As this pest would be controlled by *Bt*-maize, this herbivore is irrelevant for the present case study. Other widespread maize pests include wireworm larvae (*Agriotes* spp.; Coleoptera: Elateridae), frit fly larvae (*Oscinella frit*; Diptera: Chloropidae) and larvae of the black cutworm (*Agrotis ipsilon*; Lepidoptera: Noctuidae). Other herbivores that are regularly found in maize and are potential pests include: various cereal aphid species (Hemiptera: Aphididae), thrips (*Frankliniella* spp.; Thysanoptera: Thripidae) and spider mites (*Tetranychus urticae*; Acarina: Tetranychidae). These herbivores, however, do not usually cause economically relevant plant damage as their populations are kept below the economic injury level (EIL) (see Definition of environmental quality) by an array of natural enemy groups (table 2) and by abiotic environmental conditions. Aphids, for example, are linked to a large complex of predators and parasitoids and represent one of the main food sources for natural enemies in maize (Schmidt et al. 2003; Hajek 2004; Karley et al. 2004; Jervis 2005; Häni et al. 2006). These include plant-dwelling larvae and adults of ladybirds (Coleoptera: Coccinellidae) and predatory bugs (e.g. *Orius* spp.; Heteroptera: Anthocoridae), larvae of hoverflies (Diptera: Syrphidae), gall midges (Diptera: Cecidomyiidae) and lacewings (Neuroptera: Chrysopidae). Parasitoid wasps (Hymenoptera, mainly Aphidiidae) occupy the same stratum and are specialized on one or several aphid species. Ground-dwelling predators such as spiders (Arachnida: Araneae), ground beetles (Coleoptera: Carabidae) and rove beetles (Coleoptera: Staphylinidae) have a much wider prey spectrum, but include aphids in their diet and are able to suppress their numbers.

Definition of environmental quality

The second step aims at defining the environmental quality to be preserved (table 1). So far the protection goal (or more precisely the assessment endpoint) had only been defined qualitatively by describing the entity (i.e. natural enemies) and the attribute (i.e. regulation of herbivore populations) to be preserved. To allow decision making by regulatory authorities, the assessment endpoint

Table 3 Definition of the environmental quality that should be preserved in the assessment endpoint 'Biological control functions of natural enemies' (adapted from Sanvido et al. 2006)

Criteria	Question	Example
Entity	What has to be surveyed?	Natural enemies
Attribute	What environmental quality should be preserved?	Functional biodiversity of natural enemies (regulation of insect pest populations)
Magnitude	To what extent the environmental quality can be affected?	Maize herbivores are kept below economic injury levels
Spatial scale	In which habitats should the environmental quality be preserved?	Maize fields in agricultural landscapes with *Bt*-maize cultivation
Temporal scale	During which period of time should the environmental quality be preserved?	No failure of biological control functions during present cropping season of maize

should be quantified as far as possible using measurable criteria (Sanvido et al. 2006). This includes defining the magnitude and both the spatial and the temporal scales relevant for the entity and the attribute to be preserved (table 3). The magnitude describes to what extent the environmental quality should be preserved (or above what threshold a change would be considered a disturbance in environmental quality). Here, the quality to be preserved was defined as a state where non-target maize herbivores remain below EILs. The EIL is defined as 'the lowest population density that will cause enough economic damage to justify the cost of additional control measures' (Pedigo et al. 1986). The EIL is a decision-making tool commonly used in integrated pest management (IPM) for the management of pests in agricultural systems (Kogan 1998). The spatial scale (i.e. the habitats in which the environmental quality should be preserved) was defined as all maize fields in agricultural landscapes with *Bt*-maize cultivation (for a detailed reasoning see Scale considerations to detect failures in biological control functions). The temporal scale (i.e. the period during which the environmental quality should be preserved) was defined as the present cropping season of maize. This is in accordance with acceptability criteria set down for regulatory testing of insecticides where non-target arthropods affected by plant protection products should be able to recover within 1 year after treatment (Candolfi et al. 2000; Anonymous 2003).

Information collection

The third step in designing the GS plan consists in designing a system to detect failures in biological control functions in maize (table 1). The system definition includes both spatial and ecological considerations as well as the definition of the methodology to be used.

Scale considerations to detect failures in biological control functions

We argue that adverse effects on natural enemies caused by the cultivation of *Bt*-maize will be detected with a higher probability in maize fields rather than outside the crop on a landscape scale, simply because the exposure of non-target arthropods to the *Bt*-toxin is the highest in maize fields and unanticipated effects can be expected to first take place on organisms present in maize fields. Moreover, changes in abundance and distribution of natural enemies possibly occurring in individual maize fields are unlikely to translate to higher scales, because these changes are partly buffered by population movements from other habitat patches. This is because, according to metapopulation ecology, landscapes can be viewed as networks of habitat patches in which species occur as discrete local populations connected by migration (Hanski 1998). It is well known that ecological functions such as biological pest control often depend on population movements between natural and cultivated areas and more precisely on colonization of arable crops by natural enemies from adjoining non-crop habitats (Duelli et al. 1990; Tscharntke et al. 2005b; Bianchi et al. 2006). Especially for most flying species, but also for surface-dwelling arthropods, turnover rates in specific habitat patches due to movements between habitats are high (Duelli et al. 1990).

A further argument to sustain a focus on the field scale relates to the aim of PMM of GM crops to provide scientific data for later decision-making processes. This requires to determine, as unambiguously as possible, the causality between detected non-target effects and the factor causing it. On a landscape level, environmental effects are influenced by a multitude of interacting factors such as the biogeographical region, landscape characteristics, habitat type and agricultural management (Tscharntke et al. 2005a; Aviron et al. 2006; Bianchi et al. 2006). All these factors influence the abundance and distribution of natural enemies. Given that the influence of the various factors could be hardly distinguishable, it could become difficult to determine unambiguously the causality between a detected unanticipated effect and the factor causing it. The likelihood to detect a relevant unanticipated effect might thus be higher in a more controlled setting focussing on the field scale with only a few factors involved than in a setting on the landscape scale involving much more complex environmental conditions.

Ecological considerations to detect failures in biological control functions

Hereafter, we will argue that direct measurements of species richness have limitations for understanding ecosystem functions. Ecosystem properties depend greatly on biodiversity in terms of functional characteristics of organisms present in the ecosystem and the distribution and abundance of those organisms over space and time (Loreau et al. 2001; Hooper et al. 2005). While theory predicts that biological control functions under different environmental conditions should be more efficient with a high diversity of natural enemies (Wilby and Thomas 2002; Tscharntke et al. 2005a; Casula et al. 2006), several studies show that adding more natural enemies to a system does not necessarily ensure higher consumption rates of prey and can actually result in the opposite due to antagonistic interactions between different natural enemy groups (Rosenheim et al. 1995; Finke and Denno 2004, 2005). Denys and Tscharntke (2002), for example, found that species diversity of predators and supposed biological control function were not correlated as predator–prey ratios did not follow the pattern of insect species richness. Species richness seems thus often to be not so important for agroecosystem function, as even only one or a few species might ensure this function (Hooper et al. 2005; Shennan 2008). This is in accordance to functional redundancy theory suggesting that some species perform similar functional roles in ecosystems so that changes in species diversity do not affect ecosystem functioning (Walker 1992; Rosenfeld 2002; Loreau 2004). Ecosystem properties may be insensitive to species loss as (i) ecosystems may have multiple species carrying out similar functional roles, (ii) some species may contribute relatively little to ecosystem properties, or (iii) properties may be primarily controlled by abiotic environmental conditions (e.g. weather events) (Hooper et al. 2005). Species richness *per se* is thus not necessarily a key element of ecosystem functioning.

Methodological approaches to detect failures in biological control functions

As demonstrated above, species richness is not necessarily a key element of ecosystem functioning. A faunistic monitoring assessing species richness and abundance of specific groups of natural enemies does thus not constitute an appropriate approach to detect local failures in biological control functions. A faunistic approach would moreover unnecessarily delay decision-making processes, given the extensive work load involved in the taxonomic identification of faunistic samples. The lengthy time periods required to analyse such data sets would thus not allow to initiate appropriate corrective measures in due time. In the present case, it appears more efficient to concentrate on functional instead of taxonomic groups to ascertain the general state of biological control functions. There are mainly two methodological approaches to assess this state. The first consists in a direct analysis via the determination of parasitation or predation rates using egg cards, sentinel hosts or prey, or life-table assessments (Luck et al. 1988; Bellows et al. 1992; Sunderland et al. 1995; Mills 1997; Jervis 2005). Because such a direct approach is generally very specific to a particular natural enemy group, it is less suited to be used in a broadly applicable surveillance programme. The second approach, we deem more appropriate, consists in an indirect analysis of the general state of biological control functions via farmer questionnaires.

Indirect approach via farmer questionnaires

The indirect approach proposed here uses unusual herbivore outbreaks as an indicator for failures in biological control functions of natural enemies. Data on pest outbreaks could be collected via questionnaires addressed at farmers growing *Bt*-maize as already practiced today in EU countries (Tinland et al. 2007). Farmers and extension services would likely be the first to notice any unusual agronomic or environmental changes as (adverse) effects will most probably emerge on or in close relation to their fields (Böttinger and Schiemann 2007). It has been recognized that such questionnaires are a useful method to collect focused PMM data on performance and impacts of the cultivation of a GM plant according to current EU legislation (Wilhelm et al. 2004; EFSA 2006; Sanvido et al. 2006). Examples for farmer questionnaires for data collection in a GS programme have been developed for a number of GM crops (Beissner et al. 2006; Schiemann et al. 2006; Böttinger and Schiemann 2007). Questionnaires for *Bt*-maize focus on potential effects related to the maize grown as well as on additional information on the cultivation methods used and on the individual on-farm situation (Schmidt et al. 2008). For comparison with conventional crop cultivation, particular emphasis is laid on the design of such questionnaires to ensure statistical validity and representativeness of the collected data (Schmidt et al. 2004; Berensmeier et al. 2006; Berensmeier and Schmidt 2007; Böttinger and Schiemann 2007).

Information analysis and evaluation

The fourth step of the GS plan consists in analysing and evaluating detected failures in biological control functions in maize (table 1). The analysis of reports on herbivore outbreaks aims at separating 'unusual' from 'usual' occurrences of maize herbivores. There are mainly three sequential steps that help to validate the significance of collected data. First, reporting by farmers' acts as a filter ensuring that reported effects have been compared to known agronomic context. Typically, farmers have a historical agronomic knowledge on the 'usual' occurrence of herbivores in their maize fields. An easily applicable trigger for increased alertness could be situations where herbivores exceed existing EILs regularly used in IPM (see Definition of environmental quality). This ensures that only unusual changes are reported that lie outside the usual variability farmers have experienced over years and that are relevant from an agronomic point of view. Second, farmer questionnaires are designed to determine whether statistically significant differences between GM and non-GM maize exist (Schiemann et al. 2006; Berensmeier and Schmidt 2007). Third, survey results indicating unusual occurrence of maize herbivores should be cross-checked with existing decision support systems for IPM, particularly with systems providing forecasts on local or regional pest infestations. Such systems help farmers to decide on appropriate pest management measures. They may either be accessible online via the internet [e.g. the German ISIP (von Kröcher and Röhrig 2007)], or available at national or regional agricultural extension services.

Aphid outbreaks could, for example, be a good indicator for disturbed biological control functions as this group of herbivores is controlled by a large variety of natural enemies (table 2). Aphid numbers can be severely depressed by predation or parasitism from a number of specialist and generalist natural enemies including syrphid and chrysopid larvae, coccinellids, carabids, spiders and hymenopteran parasitoids (Hajek 2004; Karley et al. 2004; Jervis 2005). To separate unusual from normal variation in maize herbivore infestations, one must consider life cycles, population dynamics and dispersal patterns of aphids. Aphids have an enormous capacity for population increase and aphid population densities can therefore fluctuate considerably between years and over different spatial scales (Hales et al. 1997; Karley et al. 2004). In seasons with high population densities, populations fall drastically after an exponential increase during the growth phase (Hales et al. 1997). The fact that summer aphid infestations do not multiply tremendously is a consequence of a number of biological and abiotic factors controlling aphid population increases (Karley et al. 2004). Moreover, patterns of field colonization can vary markedly over years. Wheat colonization by the grain aphid *Sitobion avenae* (Hemiptera: Aphididae), for example, revealed two different colonization patterns: 1 year was characterized by a long period of weak but continuous colonization, whereas the second year presented only one peak of colonization threefold higher than the previous year (Vialatte et al. 2007). Consequently, a single transgression of the EIL in one growing season or in a restricted geographical area would probably not be considered an unusual variation necessitating additional scientific studies. It would nevertheless necessitate further observations to determine whether a trend would become visible over several growing seasons and/or over larger geographical areas. Farmer questionnaires are able to show statistically significant differences between GM and non-GM maize. Together with pest infestation data available at regional extension services, judgements whether observed pest outbreaks represent unusual variation should be possible with sufficient certainty.

Determination of causality and decision-making

The last two steps of the GS plan consist in determining possible causalities for later decision-making processes (table 1). The establishment of causalities (step 5) and subsequent decisions (step 6) requires collaboration of both scientists and regulators.

It is important to bear in mind that any observation obtained through the described GS-approach does not allow one to directly correlate adverse effects on natural enemies to the cultivation of *Bt*-maize. The approach proposed here provides a general assessment of the state of biological control functions during transgenic maize cultivation, but it does not determine the cause of possible disturbances as a multitude of factors could be responsible.

Determining causality between the cultivation of *Bt*-maize and the unusual occurrence of a specific maize herbivore will need additional, specifically designed, scientific studies that assess specific hypotheses via an experimental or a CSM-approach. One can expect that such studies would not be completed within one growing season as evidence of the assumed adverse effect would have to be confirmed in subsequent cropping seasons and/or over larger geographical areas. Regulatory authorities would thus also have to decide whether, during the time of the investigation, the cultivation of the crop must be temporarily suspended or risk mitigation measures are necessary. Final decision-making will depend on the results of the additional studies confirming or rejecting the hypotheses of causality between *Bt*-maize cultivation and the detected failures in biological control functions. If the results suggest that causality is likely, it has to be decided whether consent for the cultivation of the investigated GM crop has to be withdrawn, risk mitigation measures have to be taken or no further action is required. Final decisions would clearly not be purely based on scientific data, but would also be influenced by economic or political considerations (Wolt and Peterson 2000; Johnson et al. 2007).

Conclusions

Biological control functions provided by natural enemies are an important protection goal that should not be affected during commercial cultivation of *Bt*-maize. Ultimately, a monitoring approach aiming at detecting failures in an ecosystem service such as biological control requires considering the theoretical basis of functional ecology. Two arguments support the fact that a faunistic monitoring of specific groups of natural enemies does not constitute an appropriate approach to detect potential failures in biological control functions in *Bt*-maize on natural enemies. First, as the Cry1Ab protein expressed in *Bt*-maize lacks toxicity on natural enemies, there is no logical hypothesis that these functions could be altered by *Bt*-maize. Second, species richness *per se* is not necessarily a key element of ecosystem functioning. Hence, analysing the general state of biological control functions in maize is a more appropriate approach to determine whether functional biodiversity is affected by the cultivation of *Bt*-maize. Failures in these functions could be surveyed indirectly by recording unusual pest outbreaks as part of GS via questionnaires addressed to farmers growing *Bt*-maize. PMM programmes do not only have to be practicable, but their results have also to be applicable for later decision-making. A major advantage of this approach is that for the first time an indicator is proposed where an applicable decision threshold (i.e. the EIL's commonly used in IPM) is at hand. This will allow regulatory authorities to take decisions within the short time periods usually available for decision-making. The approach proposed here avoids the collection of insignificant data, which cannot serve the ultimate purpose of PMM to yield a scientifically sound basis for regulatory decision-making. Although our study primarily aims at providing a monitoring approach for *Bt*-maize (Cry1Ab) being currently the only GM crop commercially cultivated in Europe, we believe that such an indirect approach could also be adopted for other types of transgenic crops.

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