

Euphytica (2008) 164:903–912
DOI 10.1007/s10681-008-9666-9

Identifying indicator species for post-release monitoring of genetically modified, herbicide resistant crops

Angelika Hilbeck · Matthias Meier ·
Armin Benzler

Received: 30 March 2007 / Accepted: 6 February 2008 / Published online: 28 February 2008
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Abstract In Europe, regulations for release and placing-on-the-market of genetically modified (GM) crops require post-release monitoring of their impact on the environment. Monitoring potential adverse effects of GM crops includes direct effects as well as indirect effects, e.g. GM crop specific changes in land and pest management. Currently, there is a gap in the pre-release risk assessments conducted for regulatory approval of GM herbicide resistant (HR) crops. Since the relevant non-selective herbicides have been registered many years ago, in current dossiers requesting regulatory approval of GM HR crops, the environmental impacts of the corresponding non-selective herbicides are either entirely omitted or the applicant simply refers to the eco-toxicological safety assessments conducted for its original pesticide approval that do not address environmental issues arising in

conjunction with the cultivation of GM HR crops. Since the ‘Farm-scale Evaluations’, it is clear that consequences for farmland biodiversity can be expected. The objective of this project was to identify relevant indicator species for the long-term impact of GM HR maize cultivation and the application of their corresponding non-selective herbicides, glyphosate and glufosinate. In this article, we describe the outcome of a modified Event Tree Analysis, essentially a funnel-like procedure allowing to reduce the large number of potentially affected non-target species to those with greatest ecological relevance and highest risk to be adversely affected based on a number of ecological criteria. This procedure allowed us to identify a total of 21 weed-Lepidoptera associations that we proposed for post release monitoring of GM HR maize in Germany.

Keywords Indicator species · Non-selective herbicides · Non-target species · Transgenic crops

A. Hilbeck (✉)

Institute of Integrative Biology, ETH Zurich,
Universitätsstrasse 16, 8092 Zurich and EcoStrat GmbH,
Hottingerstrasse 32, 8032 Zurich, Switzerland
e-mail: angelika.hilbeck@env.ethz.ch

M. Meier

Kompetenzzentrum für Sicherheit und Risikoprävention
(KSR), ZHAW Zurich University of Applied Sciences,
Jägerstrasse 2, 8401 Winterthur, Switzerland

A. Benzler

I 1.3 Monitoring, Federal Agency for Nature Conservation,
Konstantinstrasse 110, 53179 Bonn, Germany

Introduction

In Europe, regulations for release and placing-on-the-market of genetically modified (GM) crops require post-release monitoring of their impact on human health and the environment. A monitoring plan under the Directive 2001/18/EC (Annex VII) foresees ‘case-specific’ monitoring and ‘general surveillance’. Case-specific monitoring aims to refute or confirm risks

identified in the required pre-release environmental risk assessment. General surveillance aims to detect unanticipated adverse effects and long-term cumulative effects that could not be detected in pre-release testing and escaped the pre-release risk assessment. Monitoring possible adverse effects of GM crops includes direct effects as well as indirect effects, e.g. GM crop specific changes in land management (see also Council Decision 2002/811/EC, which supplements Annex VII to Directive 2001/18/EC by detailed guidance notes). However, while both are meant to be complementary, in practice, they are difficult to separate and the discussion is controversial as reporting responsibilities among different authorities and financial consequences are tied to it.

In contrast to most existing environmental monitoring programs that were typically invoked by documented damage (e.g., loss of certain species) as a reactive instrument, the monitoring of GM crops in Europe is largely a pro-active, precautionary measure. Since there is no large scale GM crop production in Europe yet, little experience exists to date with regional monitoring and long-term ecosystem impacts of GM crops. Reports on environmental effects from elsewhere are contradicting and certainly controversial (Brookes and Barfoot 2006; Garcia and Altieri 2005; Friends of the Earth 2007). In fact, when looking closer into this issue, one finds that reliable and independent data on long-term and larger scale environmental impacts of the cultivation of GM crops are scarce to non-existent globally. Over 95% of all GM crops are grown on a significant scale only in six countries in the world. Of these six countries, 53.5% are grown in the US, 17.6% in Argentina, 11.3% Brazil, 6.0% Canada, 3.7% India and 3.4% in China (James 2006). In Argentina, almost all soybeans produced today are GM HR soybean involving the application of enormous amounts of glyphosate. None of the above listed countries, however, has regional monitoring programs in place that systematically survey and collect data on the impact of these non-selective herbicides for instance on farmland biodiversity and ecosystem functioning, nor the development of resistant weeds (Heap 2007).

Hence, only few data exist to date that stem from coordinated scientific research and monitoring on the long-term environmental impact of GM HR crops. This is largely due to a gap in the current pre-release risk assessments conducted for regulatory approval of

GM HR crops. Since the relevant non-selective herbicides have been registered many years ago, in current dossiers requesting regulatory approval of GM HR crops, the environmental impacts of the corresponding non-selective herbicides are either entirely omitted or the applicant simply refers to the ecotoxicological safety assessments conducted for its original pesticide approval that, for one, can be a long time ago but, more importantly, do not address and investigate environmental issues arising in conjunction with the cultivation of GM HR crops. Before the advent of GM HR crops, non-selective herbicides were not routinely used within arable fields during the cultivation period of the crops as they would kill the crops as well. The possible impact of the cultivation of GM HR crops through the application of the corresponding non-selective herbicides on farmland biodiversity has long been recognized and, in fact, sparked the largest field trials ever conducted with herbicide resistant GM crops, the 'Farm-scale Evaluations' (FSE). The FSE largely confirmed previous predictions that at least for oilseed rape and sugar beet an additional loss of farmland biodiversity can be expected beyond and above current conventional practices. Fields were 'cleaner' due to more effective weed control, hence, less weeds left less food for their associated wildlife. For maize there did not seem to be an additional loss and some species even occurred at higher densities than in conventionally treated field, at least as long as the chosen herbicide was atrazine (Hawes et al. 2003). That herbicide, however, is banned in Europe today and with other herbicides it was challenged whether the same findings would hold (Burke 2005; Perry et al. 2004).

While recognizing, for one, the lack of data and the current controversial nature of the discussion on how to close this significant gap in pre-release risk assessments for GM HR crops and, secondly, recognizing the fact that the few data and reports existing to date give reason to believe that long-term effects on farmland biodiversity and ecological functions must be expected with increasing cultivation of GM HR crops, the German Agency for Nature Conservation as part of the national regulatory body had to take action in order to fulfil its legal mandate and responsibility to protect the biodiversity in Germany. The Agency launched a research and development project and tasked us to identify indicator species that would allow to systematically monitor the long-term,

regional impact of the cultivation of GM HR crops arising through the application of their corresponding non-selective herbicides in the agro-ecosystem. The focus was on insect indicator species. This choice was also based on the FSE findings that confirmed that larger animals such as birds and small mammals while often being a target for conservation efforts, are quite difficult to monitor due to their habitat requirements largely exceeded crop field sizes. But they all are reliant either on certain farmland plants or arthropods associated with these farmland plants for food. Following the argument of the FSE, if indicator species at that level can be identified, it is expected that this can be extrapolated to higher trophic level organisms and indicate potential effects at the top end (Burke 2003).

Methodology

As risk identification and assessment of the repeated and large scale application of non-selective herbicides during the crop's cultivation period are currently missing in the risk assessment parts of the dossiers submitted for regulatory approval which could serve as starting point for the development of a monitoring program, we had to firstly identify the potential risks and their impact pathways. This subsequently allowed us to determine suitable candidate species for monitoring that would indicate whether or not a particular potential risk actually occurs. Starting point are cause-and-effect chains of the potential environmental impacts of GM HR crops triggered by the application of their corresponding non-selective herbicides. To do that, we adopted and modified the well-known risk analysis tools called 'Event Tree Analysis' and 'Fault Tree Analysis'. Fault and Event Tree Analyses are complementary tools used in risk assessment that were originally developed by engineers identifying critical steps in complex engineering processes, e.g. aviation or large scale industrial production facilities. For environmental purposes and ecological systems, before us, also Hayes (1998) used these tools for marine ecosystems but also to identify hazards of GM HR oilseed rape in Australia (Hayes et al. 2004). Both were also proposed as valuable tools for risk assessment of GM crops by the US National Research Council (National Research Council 2002).

Fault trees are 'top-down' risk analysis tools where the analyst specifies a failure event (i.e. 'top-event') and then by combining logical functions such as 'and' and 'or', identifies all events that can or must contribute to the specified failure (Hayes et al. 2004). An Event Tree is the complementary 'bottom-up' approach where an analyst specifies an 'initiating event' and lays out the logical chain of events that can occur and lead to a number of possible consequences. Both tools yield more or less complex tree-like charts where each event chain forms one branch of the tree. They do graphically model all of the parallel and sequential combinations of events that can lead to a particular 'top event' or arise from a particular 'initiating event'. This structured, logical approach allows to rigorously evaluate the potential of these events to occur. It is based on scientific data and expert knowledge and identifies what data and information is necessary to determine reliably the outcome and the gaps of knowledge associated with the possible events in a transparent manner. Both tools provide a fairly good understanding of the reliability of the analysis and the involved uncertainties and identify research priorities for closing the most critical data gaps.

In this paper, we report about the use and outcome of a modified Event Tree Analysis that allowed us to model the potential risks arising from the application of non-selective herbicides and identify plant and insect species that most likely will be affected and are suitable to indicate within a monitoring program whether these potential risks occur in the field. A GM HR maize variety expressing resistance to either one of the two non-selective herbicides 'Glyphosate' and 'Glufosinate' served as the model GM crop.

Results

We firstly identified the potential risks and described the pathways how they could realize. This was followed by the actual analysis. The Event Tree is depicted in Fig. 1 and the results are summarized in Fig. 2. In essence, it serves as funnel-like procedure that reduces the number of possible non-target species systematically and in a transparent fashion to a number that is in practical terms feasible and that are ecologically meaningful.

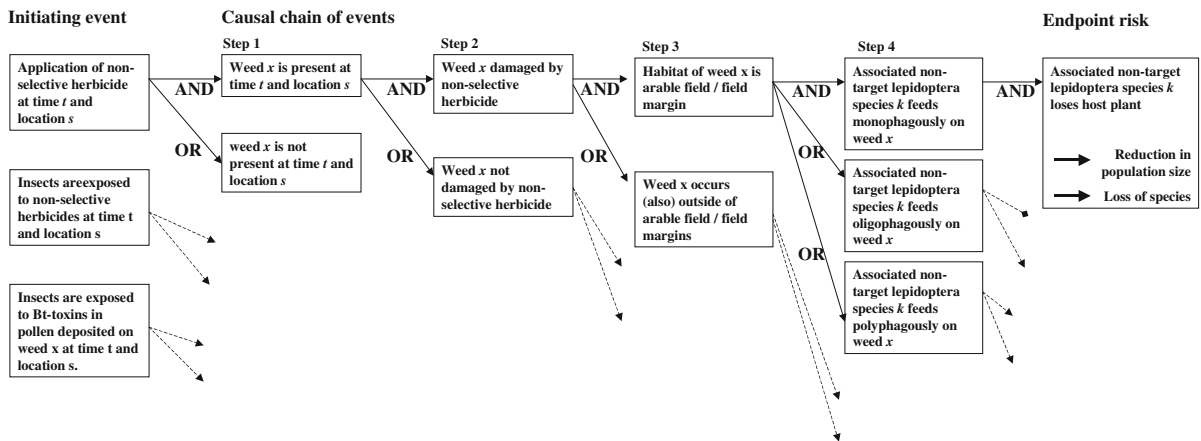


Fig. 1 Event Tree for analysing the potential risks arising from the application of non-selective herbicides when growing genetically modified, herbicide resistant maize on farmland biodiversity

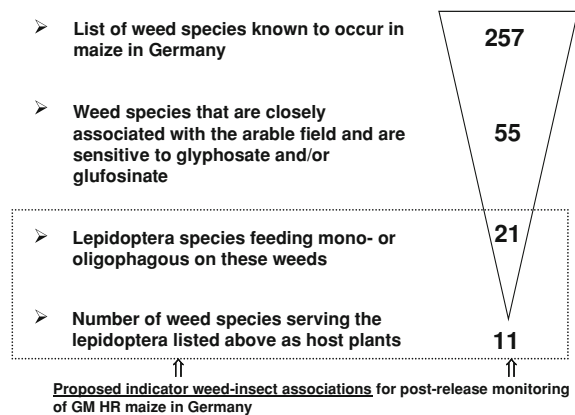


Fig. 2 Summary of the outcome of an Event-Tree Analysis for identification of weed-Lepidoptera associations as indicators for a monitoring program of genetically modified, herbicide resistant maize

Potential risk identification and impact pathways

The ‘Initiating Event’ is the application of a non-selective herbicide such as Glyphosate or Glufosinate. The application of such a non-selective herbicide intends to reduce or eliminate any plant other than the crop in the field. Hence, most if not all plant species of a maize field and its field margins will be affected certainly to some degree. Spray drift of herbicides also varies depending on the height of the spray beams. Since with GM HR crops, post-emergence application during the growing period is possible, the heights of spray beams can be expected to be higher than in conventional fields. If at all used conventionally

in arable fields, non-selective herbicides were only applied on bare soils prior to seedling emergence. Thus, also damage from spray drift to neighboring nontarget vegetation will likely be higher for GM HR crop production. Therefore, as the primary location of impact, the arable field and the habitats in the near surrounding were identified.

The primary concerns with GM HR crops and the application of their corresponding non-selective herbicides are the reduction in density and diversity of weed species, in excess to those induced by the regular herbicide regimes in conventional crops, and a shift in weed species composition towards more robust and less sensitive weed species that carry a high risk of developing resistance. This would constitute a further undesired intensification in agricultural habitats. Weed shifts and the development of resistant weeds has been described in GM HR crops for North America (van Gessel 2001). In fact, since the introduction of GM HR crops in the mid nineties, reports of glyphosate-resistant weeds stem almost exclusively from countries that grow glyphosate resistant GM crops (Heap 2007). From no reports of glyphosate resistant weeds in 1995, this number has now risen to 12, including some quite problematic weed species such as horseweed (*Conyza canadiensis*) in soybean production in the US. This predicted and troublesome development has very recently motivated Monsanto company, the largest marketing company of glyphosate and its resistant GM crops worldwide, to respond by establishing a web-based, questionnaire-driven ‘weed resistance risk assessment program’ (www.weedtool.com) for growers who use

glyphosate resistant GM crops. Along with the reduction in density and diversity of weed species a decline of the populations of nontarget arthropod species associated with these weeds was predicted and in part confirmed by the FSE (Hawes et al. 2003). Both, the weeds and their associated fauna are an important—if not the only—food source for many farmland birds and small mammals. Hence, their abundance is likely to be significantly positively correlated to the density and diversity of their primary food sources, weeds and insects. But they are much more difficult to monitor due to their large habitat requirements, high mobility and long generation times. Therefore, our focus was on finding indicator species at lower taxonomic orders that can be monitored with a reasonable amount of time and funding. Similarly, also a longer term weed shift can be indicated by the presence or absence of their associated insect fauna. The most likely affected organisms will be those weed and insect species occurring in the arable field and their surrounding habitats.

Identification of indicator species

Following from the above identified primary areas of expected impact, in the first step of the Event Tree analysis (Fig. 1), we identified to the best of our knowledge and data available the weed species that are known to occur within maize crop fields during the time of cultivation and application of herbicides (Step 1, see Fig. 1). These were compiled into a comprehensive list containing 257 weed species from 40 plant families (full list in Meier and Hilbeck 2005). In order to select those weed species whose populations would be at greatest risk of being locally eliminated and, thus, whose regional abundance and population size would be directly linked to the spatio-temporal scale of GM HR maize cultivation, the listed species were subjected to a ranking procedure. In Steps 2 and 3, the weed species were ranked according to their known sensitivity towards the non-selective herbicides (=adverse effect) and the strength of their association with certain biotope types (=likelihood of exposure/experiencing the identified adverse effect) (Fig. 1).

Efficacy of non-selective herbicides (adverse effect) (Step 2)

Based on producers information for Glufosinate, for 88 tested weed species of the above described weed

species list good or medium control of Glufosinate was reported. For glyphosate, we found information on 119 tested weed species. Glyphosate was reported to have low to some to good efficacy against 113 weed species and no efficacy against 6 species. One of the six unaffected species was *Trifolium repens* which can be controlled by Glufosinate. Against 70 of the 113 weed species that are well controlled by Glyphosate also Glufosinate works well. For the remaining 43 species no information on susceptibility to Glufosinate was found.

Association with biotope types (=likelihood of exposure/experiencing the identified adverse effect) (Step 3)

In principle, all 257 weed species listed for maize fields in Germany can be exposed to either one or both of the two non-selective herbicides used for most of today's GM HR crops. However, the overall impact on their populations on a regional scale will differ depending on their degree of association with the arable field and surrounding habitats. The closer associated a weed species is with the arable field (i.e. large proportion of a particular weed species population occurs only within the boundaries of the arable field), the closer correlated will the abundance and density of the whole population of that weed species be with the frequency and spatial scale of the application of the particular non-selective herbicide. In order to filter out those weed species that are most at risk because a significant proportion of their population or all of it occur only in the cropped field or in adjacent non-cultivated habitats, all weed species were ranked according to their association with the two critical habitat types 'agroecosystem' and the 'arable field' (see Box 1 and Table 1). For information about the biological attributes of the weed species and the association with certain habitat types see Haeupler and Muer 2000.

Further, all weed species were grouped in one of three categories combining the two classes 'agroecosystem' and 'agricultural field' to get a rough estimate of their risk to be adversely affected through the application of the non-selective herbicides (Table 1).

Of the total weed species list comprising 257 species, 81 weed species were found to be only weakly associated with the agroecosystem and the agricultural field and therefore be at lower risk to experience

Box 1: Association with 'agroecosystems' and 'arable fields'

A) Association with 'agroecosystem': this includes various habitat types belonging to the agricultural environment: different types of arable fields, pastures, grassland, meadows, unmanaged strips or lanes along small ditches, shrub thickets, but also orchards (extensively or intensively managed), abandoned areas, fallows, or unpaved field ways.

Ranking:

Close association (rank 1) – Weed species occurs only in habitat types within agroecosystems

Medium association (rank 2) – Weed species occurs at least in 1 habitat type outside of agroecosystems but in less habitat types outside than inside agroecosystems

Weak association (rank 3) – Weed species occurs in more habitat types outside than inside the agroecosystem

B) Association with 'arable field': this includes the cropped area and adjacent structures, e.g. field margins (incl. managed hedges) or unpaved field ways.

Ranking:

Close association (rank 1) – Weed species occurs only in cropped area (arable field) and potentially in unpaved field roads (similar disturbed biotope type)

Medium association (rank 2) – Weed species occurs in cropped area but also other structures listed above or exclusively in unpaved field roads

Weak association (rank 3) – Weed species hardly occurs in the cropped area but mainly in other habitat types along cropped areas (e.g. field margins, hedges, forest margins,)

Table 1 Combining and grouping species according to their association with the agroecosystem and the arable fields

Rank	Agroecosystem		
	1	2	3
1	High risk	High risk	Medium risk
2	High risk	Medium risk	Low risk
3	Medium risk	Low risk	Low risk

a significant decline in their populations. This is because many populations also occur and survive outside of the most affected areas. For another 118 weed species, a medium association (=medium risk) was concluded and only 55 weed species were found to be closely associated with both the arable field and the agroecosystem. Therefore, their population density and abundance is driven by the agricultural measures applied in the arable field (Fig. 2). These include the weed species whose populations will be at high risk of experiencing a regional decline directly correlated

to the scale of the application of non-selective herbicides during the growing period.

In a synthesis step of the procedure, we determined the cross section (logically connecting two causal events: presence at location s and sensitive to the herbicide applied at time t) of both the association with habitat type and sensitivity to either one or both non-selective herbicides most commonly used in conjunction with GM HR crops, glyphosate and glufosinate (Table 2). This allowed us to create risk categories and list the relevant species that must be considered for the next steps in the analysis.

This procedure allowed us to identify a total of 55 'high risk' weed species (see Table 3) whose population's fate will be closely correlated with the spatio-temporal scale of application of non-selective herbicides in maize fields in Germany and, thus, can indicate the intensity and effectiveness of a region-wide cultivation of GM HR crops employing either Glyphosate or Glufosinate-based non-selective herbicides.

Table 2 Risk categories when combining association with habitat and sensitivity to herbicides glyphosate and glufosinate

Herbicide	Association group		
	Close (55 species)	Medium (118 species)	Weak (81 species)
Sensitive	25 glyphosate; 21 glufosinate; 17 both (high risk)	50 glyphosate; 48 glufosinate; 37 both (medium risk)	36 glyphosate; 19 glufosinate; 16 both (low risk)
Not sensitive	0	1 glyphosate	5 glyphosate
Unknown	26	57	42

Table 3 Remaining high risk weed species (high sensitivity to non-selective herbicides, occur predominantly within arable fields or in their close surroundings, likely affected by spray drift)

Latin name	Family
<i>Rumex crispus</i> L.	Polygonaceae
<i>Viola arvensis</i> Murray	Violaceae
<i>Fallopia convolvulus</i> (L.) A. Löwe syn. <i>Polygonum convolvulus</i> L.	Polygonaceae
<i>Sonchus arvensis</i> L. ssp. <i>arvensis</i>	Asteraceae
<i>Vicia angustifolia</i> L. ssp. <i>segetalis</i> (Thuill.) Corb.	Fabaceae
<i>Vicia tetrasperma</i> (L.) Schreb.	Fabaceae
<i>Vicia villosa</i> Roth ssp. <i>villosa</i>	Fabaceae
<i>Myosotis arvensis</i> (L.) Hill	Boraginaceae
<i>Polygonum aviculare</i> L.	Polygonaceae
<i>Alopecurus myosuroides</i> Huds.	Poaceae
<i>Anthemis arvensis</i> L.	Asteraceae
<i>Apera spica-venti</i> (L.) P. Beauv. syn <i>Agrostis spica-venti</i> (L.) P.B.	Poaceae
<i>Atriplex patula</i> L.	Chenopodiaceae
<i>Avena fatua</i> L.	Poaceae
<i>Digitaria sanguinalis</i> (L.) Scop.	Poaceae
<i>Senecio vulgaris</i> L.	Asteraceae
<i>Setaria pumila</i> (Poir.) Roem. & Schult. syn <i>Setaria glauca</i>	Poaceae
<i>Anthemis austriaca</i> Jacq.	Asteraceae
<i>Spergula arvensis</i> L. ssp. <i>arvensis</i>	Caryophyllaceae
<i>Tripleurospermum perforatum</i> (Merat) Lainz syn <i>Matricaria perforata</i> , <i>Matricaria inodora</i>	Asteraceae
<i>Vicia sativa</i> L.	Fabaceae
<i>Mentha arvensis</i> L.	Lamiaceae
<i>Lathyrus tuberosus</i> L.	Fabaceae
<i>Ranunculus arvensis</i> L.	Ranunculaceae
<i>Arabidopsis thaliana</i> (L.) Heynh.	Brassicaceae
<i>Chrysanthemum segetum</i> L.	Asteraceae
<i>Fumaria parviflora</i> Lam.	Fumariaceae
<i>Fumaria schleicheri</i> Soy.-Will.	Fumariaceae
<i>Papaver rhoeas</i> L.	Papaveraceae
<i>Adonis aestivalis</i> L.	Ranunculaceae
<i>Adonis flammea</i> Jacq.	Ranunculaceae
<i>Althaea hirsuta</i> L.	Malvaceae
<i>Anthriscus sylvestris</i> (L.) Hoffm.	Apiaceae
<i>Brassica rapa</i> L.	Brassicaceae
<i>Calendula arvensis</i> L.	Asteraceae
<i>Consolida regalis</i> Gray	Ranunculaceae
<i>Crepis sectosa</i>	Asteraceae

Table 3 continued

Latin name	Family
<i>Cuscuta epilinum</i> Weihe	Convolvulaceae
<i>Diplotaxis muralis</i> (L.) DC.	Brassicaceae
<i>Euphorbia exigua</i> L.	Euphorbiaceae
<i>Geranium rotundifolium</i> L.	Geraniaceae
<i>Iberis amara</i> L.	Brassicaceae
<i>Legousia hybrida</i> (L.) Delarbe	Campanulaceae
<i>Legousia speculum-veneris</i> (L.) Chaix	Campanulaceae
<i>Linaria arvensis</i> (L.) Desf.	Scrophulariaceae
<i>Lithospermum arvense</i> L. ssp. <i>arvense</i> syn <i>Buglossoides arvensis</i> (L.) I.M.Johnston	Boraginaceae
<i>Myosotis discolor</i> Pers.	Boraginaceae
<i>Neslia paniculata</i> (L.) Desv. ssp. <i>paniculata</i>	Brassicaceae
<i>Ornithopus perpusillus</i> L.	Fabaceae
<i>Pastinaca sativa</i> L. ssp. <i>sativa</i> var <i>pratensis</i> & ssp. <i>urens</i> (Req ex Godr.) Celak.	Apiaceae
<i>Rapistrum rugosum</i> (L.) All. ssp. <i>orientale</i>	Brassicaceae
<i>Rorippa palustris</i> (L.) Besser syn <i>Rorippa islandica</i> (Oeder) Borbás	Brassicaceae
<i>Scleranthus annuus</i> L.	Caryophyllaceae
<i>Silene dichotoma</i> Ehrh.	Caryophyllaceae
<i>Silene noctiflora</i> L.	Caryophyllaceae

Identification of associated nontarget Lepidoptera species potentially at risk (Step 4)

For the remaining medium (see list in Meier and Hilbeck 2005) and high risk (Table 3) weed species, the associated Lepidoptera fauna reported to utilize them as host plants was identified and classified according to their feeding preference (Table 4). Monophagous associated species are considered to be at high risk for adverse effects if they rely exclusively on a high or medium risk host plant. Similarly, also an oligophagous associated species is considered at high risk if it feeds at a high risk host plant since its choice for finding the other one or two alternative host plants are rather slim in particular as these also must be expected to be at least locally eliminated. On the other hand, a polyphagous species feeding on a high or medium risk host plant can be expected to experience itself only a medium to low risk, respectively, as it has alternatives around. Likewise an oligophagous

Table 4 Weed-Lepidoptera associations proposed for region-wide monitoring programs of GM HR maize (combining high and medium risk weeds with feeding preference of associated fauna (here: Lepidoptera))

Risk category	Feeding preference				
	Monophagous		Oligophagous		Polyphagous
High	High risk (3 species on 3 weeds)		High risk (14 species on 9 weeds)		Medium risk
	Lepidoptera	Weed sp.	Lepidoptera	Weed sp.	A total of 3 species on 3 weeds (see Meier and Hilbeck (2005) for details)
	<i>Issoria lathonia</i>	<i>Viola arvensis</i>	<i>Argynnis adippe</i>	<i>Viola arvensis</i>	
	<i>Lycaena hippothoe</i>	<i>Rumex crispus</i>	<i>Cucullia chamomillae</i>	<i>Anthemis arvensis</i>	
				<i>Tripleurospermum perforatum</i>	
	<i>Lythria purpuraria</i>	<i>Polygonum aviculare</i>	<i>Cucullia lucifuga</i>	<i>Sonchus arvensis</i> ssp. <i>arvensis</i>	
			<i>Cucullia umbratica</i>	<i>Sonchus arvensis</i> ssp. <i>arvensis</i>	
			<i>Dypterygia scabriuscula</i>	<i>Polygonum convolvulus</i>	
				<i>Polygonum aviculare</i>	
				<i>Rumex crispus</i>	
			<i>Eupithecia virgaureata</i>	<i>Senecio vulgaris</i>	
			<i>Hecatera bicolorata</i>	<i>Sonchus arvensis</i> ssp. <i>arvensis</i>	
			<i>Lycaena dispar</i>	<i>Rumex crispus</i>	
			<i>Lycaena phlaeas</i>	<i>Rumex crispus</i>	
			<i>Pyropteron chrysidiformis</i>	<i>Rumex crispus</i>	
		<i>Tyria jacobaeae</i>	<i>Senecio vulgaris</i>		
		<i>Leptidea sinapis</i>	<i>Lathyrus tuberosus</i>		
		<i>Lygephila viciae</i>	<i>Lathyrus tuberosus</i>		
		<i>Zygaena lonicerae</i>	<i>Lathyrus tuberosus</i>		
Medium	High risk (4 species on 2 weeds)		Medium		Low risk
	Lepidoptera	Weed	A total of 45 species on 8 weeds (see Meier and Hilbeck (2005) for details)		A total of 3 species on 13 weeds (see Meier and Hilbeck (2005) for details)
	<i>Aedia funesta</i>	<i>Convolvulus arvensis</i>			
	<i>Emmelia trabealis</i>	<i>Convolvulus arvensis</i>			
	<i>Tyta luctuosa</i>	<i>Convolvulus arvensis</i>			
	<i>Lycaena hippotoe</i>	<i>Rumex acetosa</i>			

associated species feeding at a medium risk host plant is considered to experience a medium risk for its regional abundance.

This final step yielded a total of 21 Lepidoptera species whose population dynamics will be strongly influenced by the spatio-temporal scale of cultivation of GM HR crops and the application of their corresponding herbicides. The identified 21 high risk lepidoptera species are all species that are highly dependent on 11 weed species. For all of these weed species a negative correlation of their abundances and densities locally and regionally with the spatio-temporal scale of cultivation of GM HR crops, beyond and above of what these weed species experience

today under conventional weed control regimes, must be expected. However, as little to no data from long-term surveillance programs is available on the region-wide impacts of the large scale and repeated application of non-selective herbicides and, as currently practised pre-approval risk assessment omits these adverse effects (see Introduction), we propose to monitor selected weed-Lepidoptera associations rather than their individual components (i.e. either weed species or associated lepidopteran species). Certainly until sufficient evidence and experience has been gained that would allow to possibly monitor only the host plant (i.e. weed) or the corresponding associated species by itself.

Summarizing conclusions

It is widely known that agricultural habitats presently contribute significantly to biodiversity in terms of species richness at the European level. At the same time farming practices have intensified rapidly, leading to a dramatic loss of biodiversity in agricultural areas (Hoozeveen et al. 2002). Today, many characteristic species of agricultural systems feature on Europe's red lists of endangered species. At the EU level, agri-environment policies have been introduced as a promising policy tool to reverse the negative biodiversity trend. Preserving low intensity farming and preventing further intensification should have top priority (Hoozeveen et al. 2002). Therefore, in their report commissioned by the Council of Europe, the French Government and the United Nations Environment Program, Hoozeveen et al. (2002) concluded that special conservation efforts for preserving agricultural biodiversity are justified. This and relevant European environmental legislation forces the competent authority on nature conservation in Germany to act on the issue of GM HR crops in German agriculture. In this article, we described the outcome of a modified Event Tree Analysis essentially a funnel-like procedure allowing to reduce the large number of potentially affected non-target species to those with greatest ecological relevance and highest risk to be adversely affected based on a number of ecological criteria (Fig. 2). A total of 21 weed-Lepidoptera associations were identified and proposed for monitoring.

However, the outcome of the selection procedure could still be developed further by including other ecological aspects that might modulate the final outcome. Some associated species considered to be at lower risk might experience a higher degree risk of local extinction than thought depending on their mobility and location of hatch. For instance, even if highly polyphagous, caterpillars in a large maize field sprayed with glyphosate will have to walk long distances before finding suitable alternative host plants outside of the cropped field again, possibly too long, depending where they hatch and have to start their journey. However, their overall population might be in a better position to compensate for such losses, if these losses remain localized.

Another issue is if certain weed-Lepidoptera associations occur also in other GM HR crops. For

example, *Issoria lathonia* is reported to feed monophagously on *Viola arvensis*. While *V. arvensis* does occur in maize fields, it has its peak density and is most problematic as a weed in oilseed rape where most herbicides used today have a gap of efficacy. Hence, today, they occur in conventional oilseed rape in high and very high densities. Both non-selective herbicides, glyphosate and glufosinate, control this weed well. Therefore, in the case of GM HR oilseed rape cultivation, *I. lathonia* will almost inevitably experience a serious if not locally complete loss of host plants which can likely affect its regional population densities and abundances. This particular species will be under great pressure when both HR maize and HR oilseed rape will be grown. An additional threat will also constitute the Bt-containing pollen from the stacked GM HR/Bt maize varieties that are being increasingly used (James 2006). Therefore, the *Viola arvensis*-*Issoria lathonia* association was scrutinized further using the complementary Fault Tree Analysis where the loss of both species are the identified 'top event' (Meier and Hilbeck 2005).

All of the above identified 21 weed-Lepidoptera associations are in principle suited for post-release monitoring programs. However, it might not be necessary to monitor all of them. Further reduction efforts could for example focus on locally most common weed-Lepidoptera associations. Monitoring monophagous species has the advantage that these species will represent quite sensitive indicator species as they are dependent on one host plant and will reflect their density and abundance without much delay. On the other hand, they might over-proportionally reflect heterogenous distribution patterns of their host plants that are independent of the herbicide use intensity. Oligophagous species could buffer this better but might be less sensitive to intensity of the usage of non-selective herbicides. Therefore, we propose to include a variety of locally existing monophagous and oligophagous weed-Lepidoptera associations in a regional monitoring program.

Risk analysis driven identification of indicator species has proven useful in particular in face of lacking pre-release risk assessments and post-release field data. It allows to select indicator species that are ecologically meaningful and sensitive to the anticipated impacts caused by the introduction of GM HR crops in a transparent and scientifically logical manner. While we conducted this procedure using GM HR maize as the case example, this could be done similarly with any GM

crop. This project was a significant step forward towards the identification of a reduced list of sensitive indicator species for biodiversity impact of GM HR crop plant production but more efforts need to go into their fine-tuning to regional conditions. This could potentially allow to further reduce the investments necessary for the monitoring but yet maintain its indicative power. Further, sampling strategies and test runs must now be carried out to have the necessary methodologies ready when large scale GM HR crop production begins in Germany.

Acknowledgements This work was supported by the German Federal Agency for Nature Conservation with funds of the German Ministry for Environment, Nature Conservation and Nuclear Safety.

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