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GRACE

Reviews of Environmental Impacts of GM Plants - Conceptual Models and Review Questions

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GRACE
GMO Risk Assessment and Communication of Evidence

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Review of environmental impacts of GM plants

1 Motivation

Genetically modified (GM) crops have been approved for commercialisation following risk assessments and have been cultivated in several countries for up to 20 years. The original risk assessments identified a few environmental risks which are being managed in some GM crops. In most cases GM crops have replaced existing crop varieties. However, in some cases, GM crops have allowed novel cultivation of the crop in some regions for various agronomic or economic reasons. A major concern raised by stakeholders and regulators is whether there is now evidence that GM crops and their cultivation are causing environmental harm compared with the crop varieties that they have replaced. We are therefore considering two major areas of concern:

- Is there evidence that GM crops and their cultivation are adversely affecting biodiversity or populations of non-target species and their associated ecosystem functions?
- Is there evidence that GM crops and their cultivation are causing the development of resistant pests and weeds?

2 Background

A major part of the regulatory process for GMOs is the environmental risk assessment, which aims at determining potential risks to the environment. For Europe, there are a range of Environmental Protection Goals which could be affected by cultivation of GM crops, including Biodiversity, sustainable agriculture, soil quality and functionality and a range of ecosystem services such as biodegradation, pollination and predation which can influence integrated pest management. The European Food Safety Authority (EFSA) lists the following specific areas of risk to be addressed (EFSA 2010): (1) Persistence and invasiveness, including plant-to-plant gene flow; (2) Potential for plant to micro-organisms gene transfer; (3) Interaction of the GM plant with target organisms; (4) Interaction of the GM plant with non-target organisms; (5) Impacts of the specific cultivation, management and harvesting techniques; (6) Effects on biochemical processes; and (7) Effects on human and animal health. In addition to the data specifically generated by the applicants for the product to be approved, relevant published data available from the scientific community is also used to support the environmental risk assessment. The relationship between the environmental interventions caused by GM crops, their areas of environmental impact in farmland ecosystems, the environmental risks they cause and how this is related to harm to environmental protection goals is described in Fig 1.

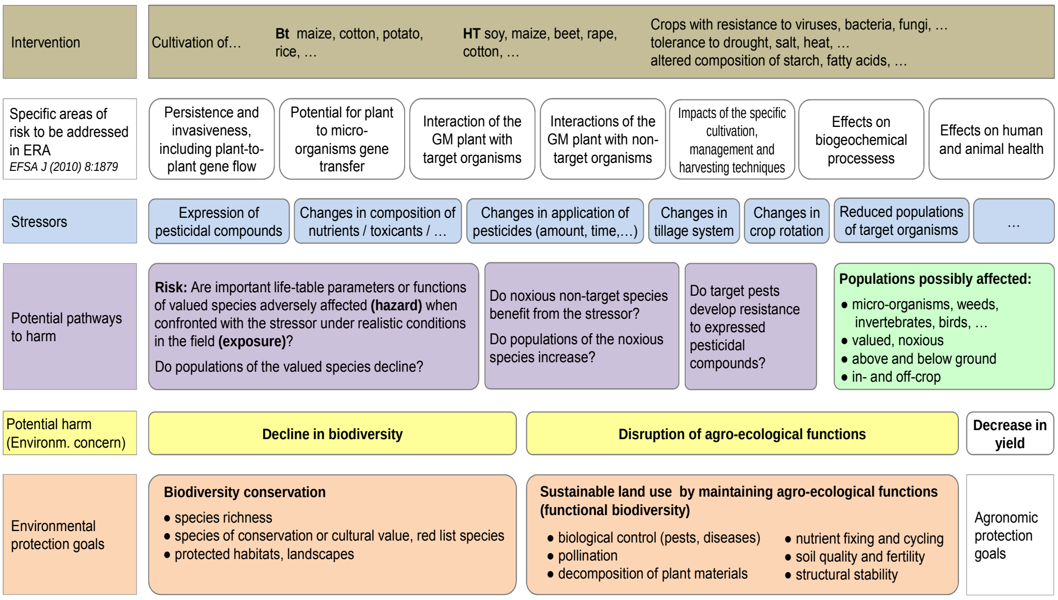


Figure 1: Conceptual model of the relationship between the intervention (cultivation of different genetically engineered traits and crops), the areas of risk to be addressed in the environmental risk assessment (ERA) according to EFSA, the potential stressors, the pathways to harm (including hazard, exposure, and risk), typical environmental concerns and typical environmental protection goals according to European legislation. The basis for this model is the EFSA ERA guidance (EFSA Journal (2010) 8: 1879).

There is considerable data from numerous lab, glasshouse and field studies of the effects of GM crops on biota associated with the cultivation of GM crops, both pre and post market. These include impacts on target and non-target species which can be directly affected by the GM plant or indirectly affected through food chain effects and by the specific management applied to GM crops. Risk assessors and risk managers need to know the conclusions and outcomes of all the available studies in order to determine whether cultivation of GM crops is having adverse environmental effects and whether these constitute environmental harm when compared with current cropping practices in Europe. In order to do this they need all the available evidence regarding potential environmental risks of GM plants in an easily accessible form and they would also benefit from a comprehensive and systematic synthesis of existing evidence including a quality assessment of data.

Objectives of the GRACE review of the evidence of environmental impacts of GMOs

This document describes the processes developed in order to conduct systematic reviews of the available information that relates to the environmental impacts of the cultivation of GM crops. It describes the conceptual models which are used to develop the rationales for selecting the review topics and for developing the review questions which are the starting points for the reviews. Stakeholders are requested to comment on the conceptual models, the rationales for selecting the review questions and on the relative importance of the review questions.

The assessment end points considered are non-target flora, fauna, and microorganism populations and selected ecological functions performed by these organisms. The reviews will collect data from these studies which will be collated and analysed in order to assess whether there is evidence that non-target organisms and functions have been affected by the GM crop and its cultivation. In addition data will be collated and analysed on the effects of Bt crops and their cultivation on resistance development in target species and on the development of weed resistance to the herbicides used in GM herbicide tolerant (HT) crop cultivation.

3 Review topics and review questions

The Review topics relate to the most widely cultivated GM crop types worldwide (Bt and HT) and the evidence on the environmental impacts of these crop types. The environmental impacts that are particularly considered are those on non-target and target species of the GM crop and its related management. The topics are described below but are not listed in any particular order of priority

3.1 Review topic 1: Impact of GM Herbicide Tolerant (GMHT) crops

HT crops are widely grown in North and South America and are being developed for many other regions including Europe. Since use of herbicides has been associated with declines in farmland biodiversity in some regions, there are concerns that GMHT crops treated with broad spectrum herbicides will also cause declines in biodiversity (Sweet and Bartsch, 2011, 2012). However many HT systems also incorporate the use of minimal tillage which also has biodiversity impacts, so that total impacts of HT systems require study in order to determine GM HT cropping effects. There are also concerns that repeated use of the herbicides used on HT crops will promote herbicide resistance development (Owen & Zelaya, 2005) and further inappropriate use of herbicides leading to reductions in biodiversity (Benbrook, 2012).

The protection goals considered in relation to GMHT crops include: farmland biodiversity, sustainable agricultural production, integrated pest management, soil quality and function (including nutrient cycling etc).

3.1.1 Review question development

Concept: Stakeholder Concern: The cultivation of HT crops will reduce biodiversity and ecosystem services in farmland regions.

Objective: Determine whether research studies and data show shifts or declines in populations of biota in GMHT compared with conventionally managed crops.
Conceptual Model: Review data from field studies of GMHT crops: compare effects of GM crop, herbicide regimes and associated management applied to HT crops and conventional crops for impacts on flora, non target invertebrate fauna and microorganism populations and selected ecological functions as assessment end points or indicators of impacts on biodiversity and ecosystem services.

3.1.2 Review questions

Review Topic 1:

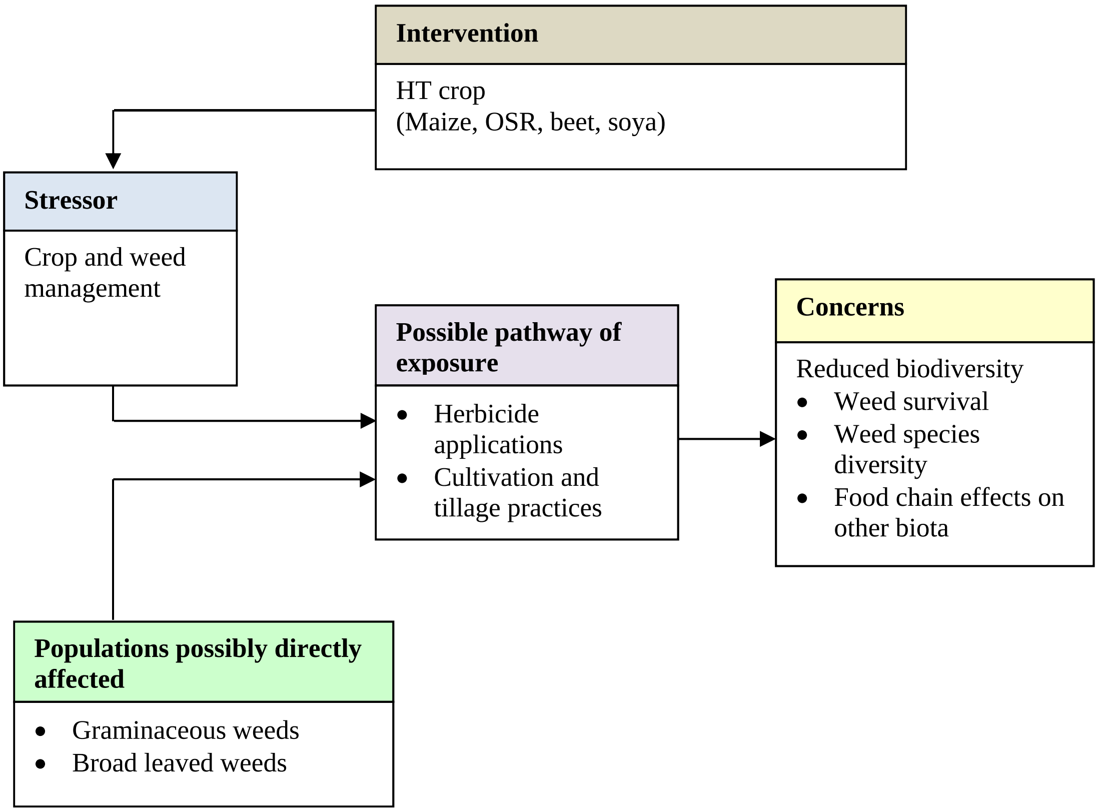


Figure 2: Conceptual Model describing potential impacts on botanical diversity caused by the herbicides and management of GM HT crops (compared with conventional crops).

Specific Review question 1:

Are weed populations (P) changed (O) by management regimes applied to GM HT crops (I) compared with conventional management (C)?

Review Topic 1.2: Effects of GMHT cropping on NT invertebrates and micro-organisms

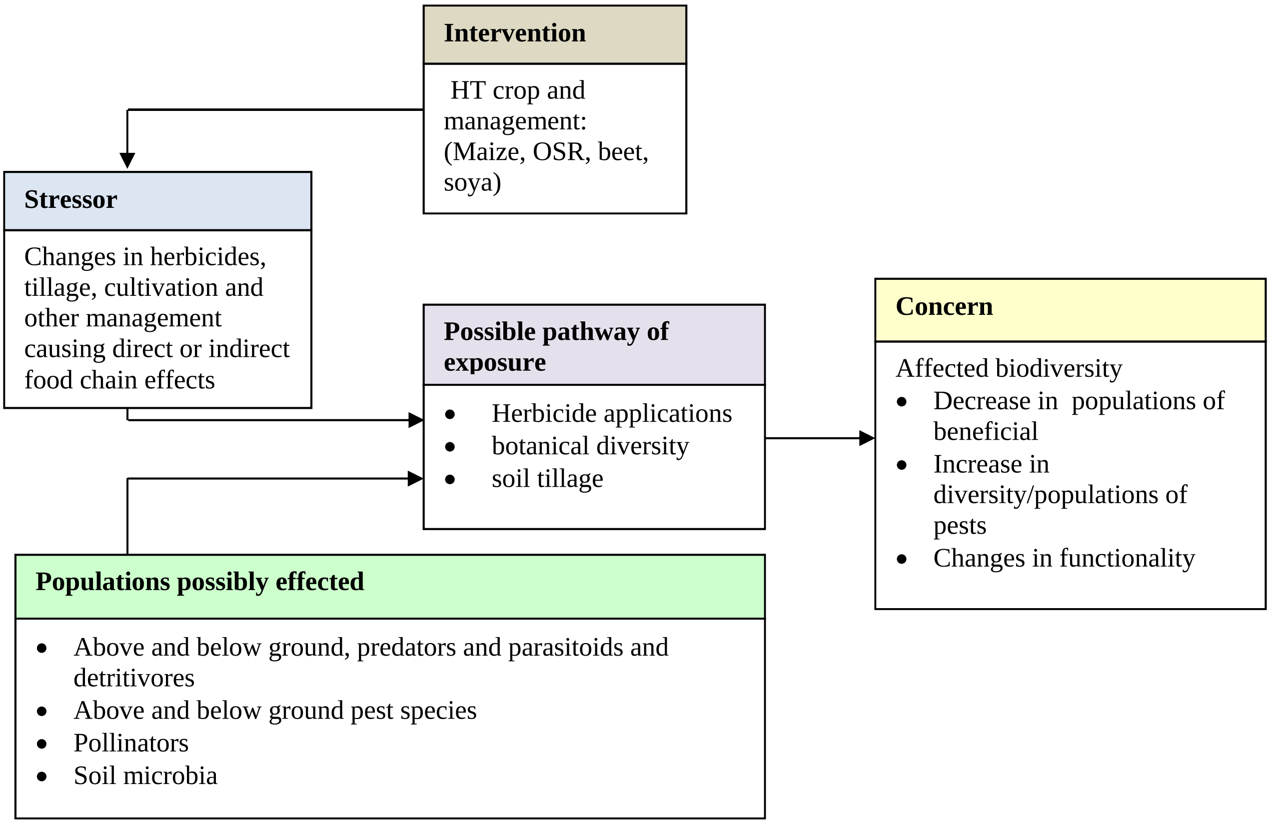


Figure 3: Conceptual Model describing potential impacts on invertebrate and microbial populations and their functions caused by management of GM HT crops compared with conventional crops.

Specific Review Question 2:

Are above ground invertebrate populations (P) changed (O) by management regimes of GM HT crops (I) compared with conventional management applied to genetically similar varieties (and/or these varieties with no herbicide treatment where relevant) (C)?

The study of soil invertebrates is below (Topic 1.4)

Review Topic 1.3: Soil microorganisms and HT cropping systems

The conceptual model in Fig 3. also applies to soil micro-organisms which may be affected by the changed herbicide programs as well as by other changes in soil management associated with GMHT crops.

The Micro-Organisms and activities of interest (P)

Soil microorganisms (basically bacteria and fungi) and their activities. Include biomass, numbers, presence of specific taxa and functional groups. Diversity as measured by different phenotypic and genotypic methodologies. Activities e.g. respiration, enzymatic, inorganic compounds, decomposition.

Type of exposure (E)

Exposure to HT crops and their concomitant farming practice through the soil environment and in the rhizosphere.

Comparator (C)

The near-isogenic crop in an experimental design allowing for any of the comparisons:

- GM HT, non-HT plots and control plots where the first two receive regimes of herbicides and optionally an additional comparator not receiving herbicides.
- Farming systems comparisons where the HT-system includes a herbicide regime as well as tillage practise contrasted to a comparator with conventional tillage and a GAP herbicide regime.

Outcome (O)

Microbial parameters contrasted with the comparator and calculated as Hedges' d for any of the comparators (?).

Specific Review question 3:

Are soil microbial endpoints (P) changed (O) by GMHT herbicide and management regimes (E) compared with conventional management (C)?

Review Topic 1.4: Soil invertebrates and HT crops

The conceptual model in Fig 3. also applies to soil invertebrates which may be affected by the changed herbicide programs as well as by other changes in soil management associated with GMHT crops.

Population of interest (P)

Soil invertebrates at species level or at higher taxonomic levels among earthworms, enchytraeids, nematodes, collembolans and mites.

Type of exposure (E)

Exposure to genetically modified HT crops and their concomitant farming practice through the soil environment and in the rhizosphere. The major environmental factors in HT cropping systems are pesticides, tillage and crop rotation, i.e. as valid for any agricultural system.

Comparator (C)

The near-isogenic crop in an experimental design allowing for any of the comparisons:

- HT, non-HT plots and control plots where the first two receives regimes of herbicides according to GAP and an additional comparator not receiving herbicides.
- Farming systems comparisons where the HT-system includes a herbicide regime as well as a tillage practice contrasted to a comparator with conventional tillage and a GAP herbicide regime.

Outcome (O)

Population abundances indv. m^{-2} or biomass m^{-2} contrasted with the comparator and calculated as Hedges' *d* for any of the comparators.

Specific Review question 4:

Are population abundances (O) of soil invertebrates (P) changed by GMHT herbicide and management regimes (E) compared with conventional management (C)?

GM HT crops: Review Topic 1.5

Concept: Stakeholder Concern: there is considerable evidence that in some regions of N and S America the cultivation of HT crops will encourage development of resistant weeds (Owen & Zelaya, 2005; Benbrook, 2012). This could lead to changed or increased use of herbicides in farmland areas with associated adverse biodiversity effects.

Objective: Determine whether studies of commercial cultivation of GM HT crops show shifts in resistance development (specifically to glyphosate) in weed populations in HT compared with conventionally managed crops and crop rotations.

Conceptual Model: Review data from studies of HT crops: compare effects of herbicides applied to HT crops and conventional crops for impacts on weed resistance development.

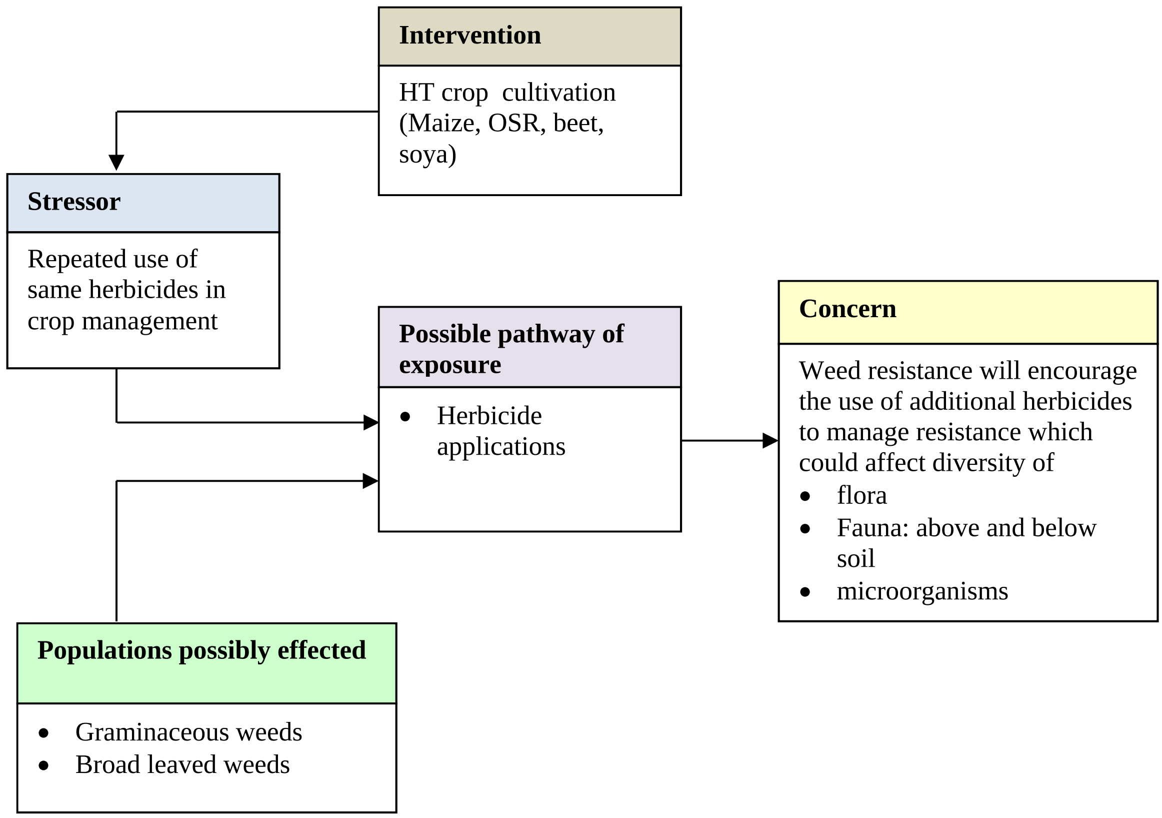


Figure 4: CM describing how potential weed resistance development in HT crops could result in further changes to applications of herbicides resulting in additional environmental impacts.

Specific Question 5:
Do weed populations (P) exposed to HT herbicide regimes (I) compared with conventional herbicide regimes (C) become more resistant to herbicides (O)?

3.1.3 List of review questions on Environmental topic 1: GM HT crop

1. Are weed populations (P) changed (O) by GMHT herbicide regimes (I) compared with conventional herbicide management (C)?
2. Are arthropod populations (P) changed (O) by GMHT herbicide and management regimes (I) compared with conventional management (C)?
3. Are soil microbial endpoints (P) changed (O) by GMHT herbicide and management regimes (E) compared with conventional management (C)?
4. Are population abundances (O) of soil invertebrates (P) changed by GMHT herbicide and management regimes (E) compared with conventional management (C)?
5.Do weed populations (P) exposed to HT herbicide regimes (I) compared with conventional herbicide regimes (C) become more resistant to herbicides (O)?

3.2 Review topic 2: Bt crops

GM maize producing Cry proteins from the bacterium *Bacillus thuringiensis* (*Bt*) is the only GM crop cultivated in Europe on significant areas for more than a decade. In 2011, it was planted on a total area of 115 000 ha in 6 European countries with Spain growing the largest area (James 2011). While MON810 targeting corn borers (Lepidoptera) is currently the event that is approved for commercial cultivation in the EU, more than a dozen events targeting the corn rootworm (Coleoptera) as well as stacked events (expressing several *Bt* proteins simultaneously) are in the regulation pipeline (Meissle et al. 2011). Because *Bt* maize is engineered to produce insecticidal proteins, potential interactions with non-target organisms (NTOs) are a major concern in risk assessments. In particular beneficial arthropods that provide important ecological services, such as pollination, decomposition, and biological control are considered.

A range of research programmes on Bt maize have been conducted in many European countries, such as Spain, Germany, Czech Republic, Hungary, Poland, and Romania. Usually, those programmes included experimental field studies and potential effects on (NTOs) were studied (Romeis et al. 2008).

Scientists in the USA have conducted meta-analyses on field data on the impact of *Bt* crops on NTOs published between 1992 and early 2008 (Marvier et al. 2007; Wolfenbarger et al. 2008; Naranjo 2009). Although those studies analyzed data on a global scale, the majority of the data that went into the meta-analyses were derived from field studies conducted in the USA.

3.2.1 Bt crops review question development

Bt Crops Review questions:

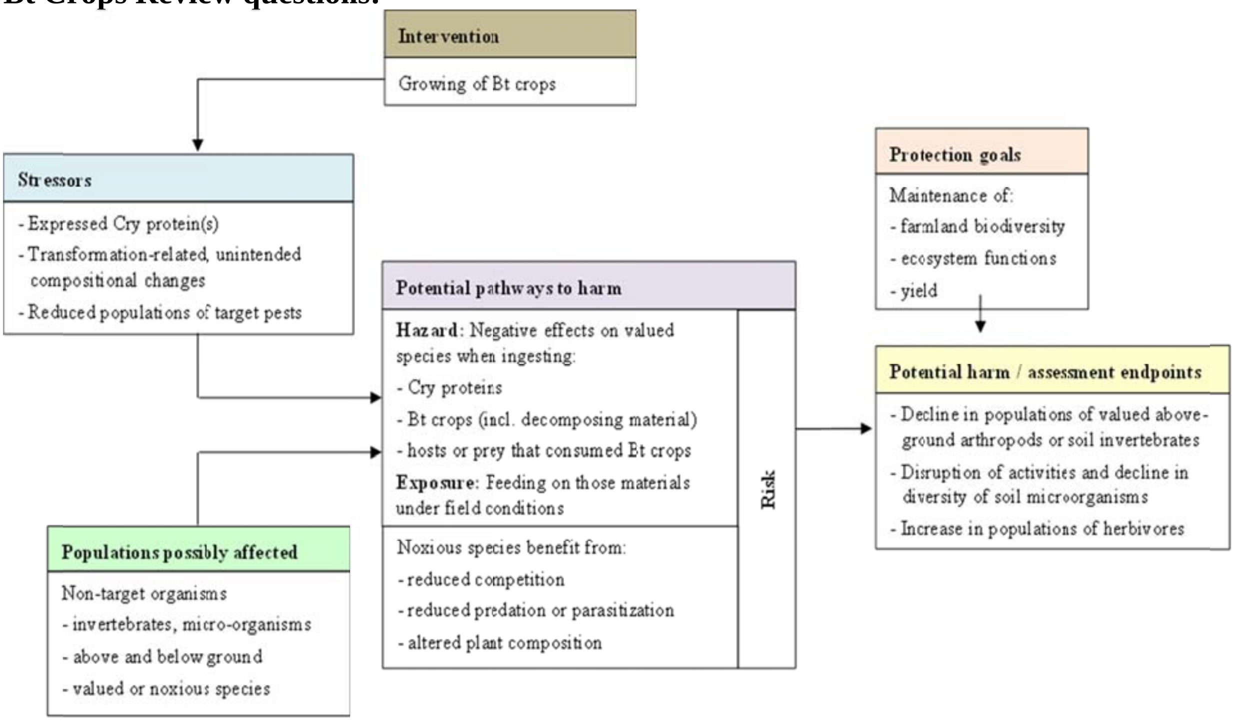


Figure 5: CM describing potential impacts on the biodiversity of NTOs caused by the cultivation of *Bt* crops

i. Above-ground non-target arthropods and Bt maize

Because of its relevance for Europe, potential effects of Bt maize on non-target arthropods will be reviewed. We identified the following potential pathways to harm: (i) the production of Bt proteins may lead to direct adverse effects on non-target arthropods; (ii) transformation-related, unintended changes in plant composition of nutrients or toxicants may lead to adverse effects on non-target arthropods; (iii) Bt proteins and/or transformation-related effects may influence hosts or prey species which triggers adverse effects on their parasitoids or predators, and (iv) noxious arthropods may benefit from reduced competition with target pests or from reduced predation or parasitization. Common to all pathways is that harm can only occur if field populations of valued non-target species decline or populations of noxious species increase. Therefore, the first systematic review focuses on populations of arthropods in Bt maize fields by answering the following question:

Review Question 1. Does the growing of Bt maize (*Intervention*) change populations or ecological functions (*Outcome*) of non-target arthropods (*Population*) compared to the growing of conventional, non-GE maize (*Comparator*)?

An evidence map will be created that identifies

(i) From which countries/ continents data are available
(ii) Which taxonomic/ functional groups of arthropods have been studied
(iii) Which parameters have been measured in field studies

Based on the knowledge gained from previous meta-analyses (Marvier et al. 2007; Wolfenbarger et al. 2008; Naranjo 2009), most data came from North America and Europe, functional groups included predators, parasitoids, herbivores, omnivores, and detritivores, and abundance was the parameter most commonly measured. Therefore, we plan to conduct more detailed data extraction and statistical meta-analyses answering the following, more specific question:

Review Question 2. Does the growing of Bt maize (*Intervention*) change the abundance (*Outcome*) of non-target arthropods in European and North American maize fields (*Population*) compared to the growing of conventional, non-GM maize (*Comparator*)?

ii. Soil invertebrates and *Bt* crops:

For organisms living in soil, we will broaden the scope of the review by including all Bt crops (not only Bt maize) and by including all invertebrates (not only arthropods). We plan to conduct the following systematic review:

Soil invertebrates and *Bt* toxins (PECO):

Population of interest (P)

Soil invertebrates at species level or at higher taxonomic levels among earthworms, enchytraeids, nematodes, collembolans and mites.

Type of exposure (E)

Exposure to genetically modified *Bt* crops and their concomitant farming practice through the soil environment and in the rhizosphere.

Comparator (C)

The near-isogenic crop in an experimental design allowing for any of the comparisons:

- *Bt* with non-*Bt* plots, neither of which received any additional insecticide treatments.
- unsprayed *Bt* plots with non-*Bt* plots that received insecticides.
- *Bt* with non-*Bt* fields when both are subject to insecticide treatments.

Outcome (O)

Population abundances indv. m^{-2} or biomass m^{-2} contrasted with the comparator and calculated as Hedges' g for any of the comparators:

$(x_E - x_C)/s$

where s is the pooled standard deviation, E is the GM treatment effect and C is the comparator.

Review question 2: Are population abundances (O) of soil invertebrates (P) changed by *Bt* crops (E) compared with conventional crops (C)?

iii. Soil microorganims and *Bt* toxins

This topic will study available data on the effects of Bt crops on soil microorganism populations (bacteria and fungi) and their functional activities such as respiration, decomposition etc to determine whether there is evidence of changes and the significance of these changes.

Organisms and activities of interest (P)

Soil microorganisms (basically bacteria and fungi) and their activities. Include biomass, numbers, presence of specific taxa and functional groups. Diversity as measured by different phenotypic and genotypic methodologies. Activities e.g. respiration, enzymatic, inorganic compounds, decomposition.

Type of exposure (E)

Exposure to genetically modified *Bt* crops and their concomitant farming practice through the soil environment and in the rhizosphere.

Comparator (C)

The near-isogenic crop in an experimental design allowing for any of the comparisons:

- *Bt* with non-*Bt* plots, neither of which received any additional insecticide treatments.
- unsprayed *Bt* plots with non-*Bt* plots that received insecticides.
- *Bt* to non-*Bt* fields when both are subject to insecticide treatments.

Outcome (O)

Microbial parameters contrasted with the comparator and calculated as Hedges' d for any of the comparators.

Review question 3:

Are soil microbial endpoints (P) changed (O) by *Bt* crops (E) compared with conventional crops (C)?

3.2.2 List of review questions topic 2: Bt Crops Non-Target Effects

Review questions
1. Does the growing of Bt maize (I) change populations or ecological functions (O) of non-target arthropods (P) compared to the growing of conventional non-GM maize (C)?
2. Does the growing of Bt maize (I) change the abundance (O) of non-target arthropods in European and North American maize fields (P) compared to the growing of conventional, non-GM maize (C)?
3. Are population abundances (O) of soil invertebrates (P) changed by *Bt* crops (E) compared with conventional crops (C)?
4. Are soil microbial endpoints (P) changed (O) by *Bt* crops (E) compared with conventional crops(C)?

3.3 Review Topic 3. Target Insect Resistance Development and Resistance Management of Bt Crops

Introduction

Lepidopteran and coleopteran species are the most important pests in maize. However, one concern with growing *Bt*-maize for control of these pests is the rapid evolution of resistance. Resistance evolution occurs regularly where pest populations are exposed to a uniform, strong and continuous selection pressure (Roush 1994, Tabashnik 1994, Andow 2008) and is therefore expected to occur in insect resistant GM plants expressing *Bt*-proteins. In consequence *Bt*-products might lose their effectiveness against pests either as conventional sprays or as transgenic traits of GM crops. Therefore resistance management must accompany the cultivation of *Bt*-crops to delay the evolution of resistance to *B. thuringiensis* products (Hokaanen and Wearing 1994).
Within the EFSA opions on *Bt*-crops, resistance evolution is identified as a potential risk and the efficacy of strategies for slowing down the resistance evolution proposed by applicants are evaluated. The most common strategy is the “high-dose/refuge” strategy (Andow 2008, Tabashnik & Gould 2012) and models are used to forecast the evolution of resistance.
The principle of the high-dose/refuge strategy used in these models are that (1) the protein is killing nearly all pest organisms feeding on GM plant (high dose), (2) the frequency of resistance alleles is low, (3) the inheritance of resistance is fully recessive, (4) rare resistant pests surviving on *Bt*-crops mate with abundant susceptible pests from nearby refuges of host plants without *Bt*-proteins (Tabashnik & Gould 2012), and (5) fitness costs are associated with the evolution of resistance. Therefore comprehensive data on the biology of the target organisms, the characteristics of the modified plant and the GM trait are needed to support the model. In the case that not all requirements for the high dose refuge strategies are fulfilled, a modified strategy or additional measures might be needed.
In the first part of the review information on European target species, whose resistance evolution might be a serious problem should be identified. For these species all relevant information to predict the evolution of resistance will be collected and collated in particular with a focus on European data.
In the second part of this review the range of suggested and modified strategies should be documented and evaluated. The search will look for failure of resistance strategies and their reasons. This might help to improve and adapt insect resistance strategies on European conditions.
The information should be collected for both lepidopteran and coleopteran maize pest species.

3.3.1 Resistance Development and Resistance Management of target organisms exposed to specific Cry proteins

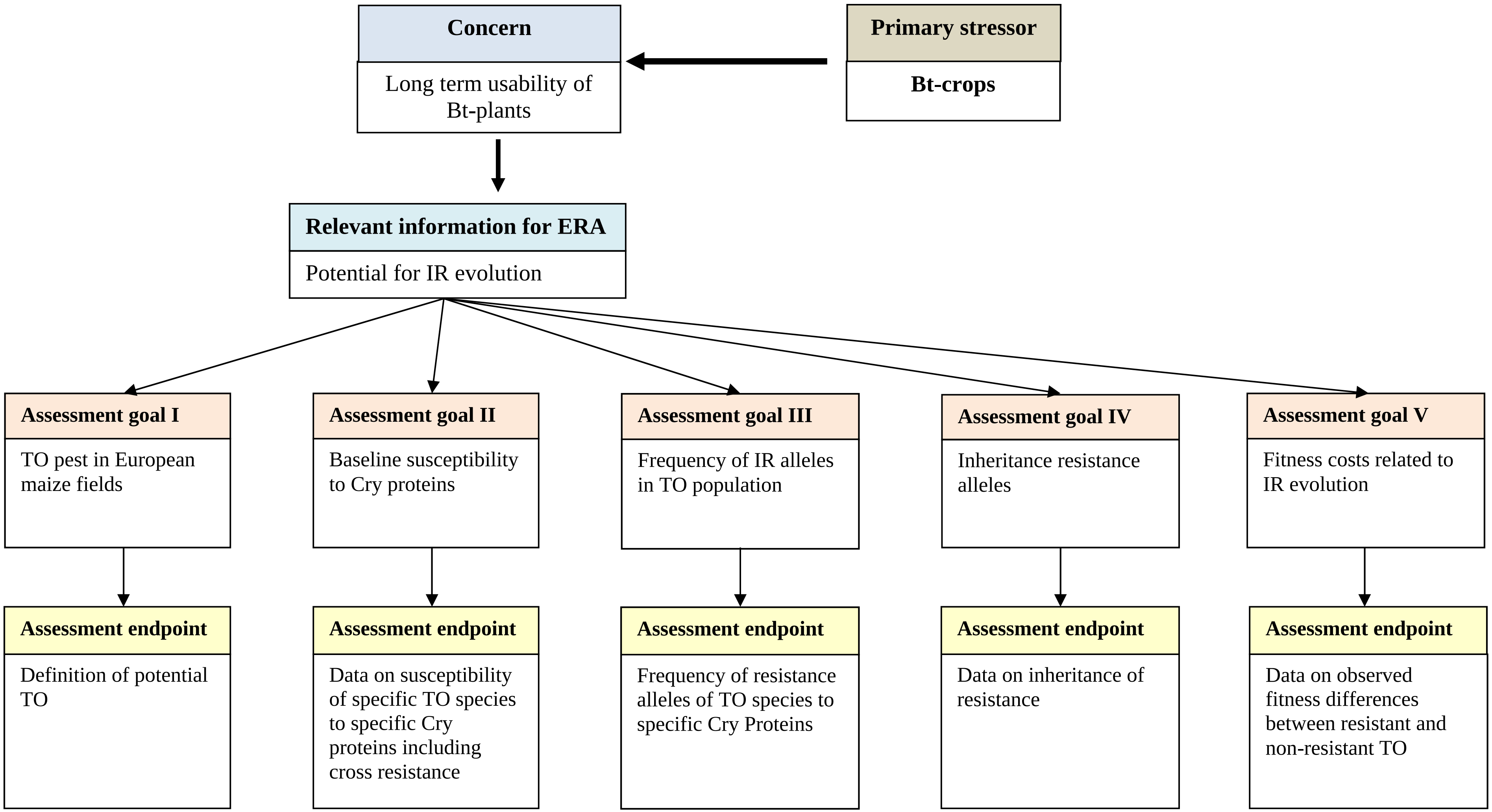
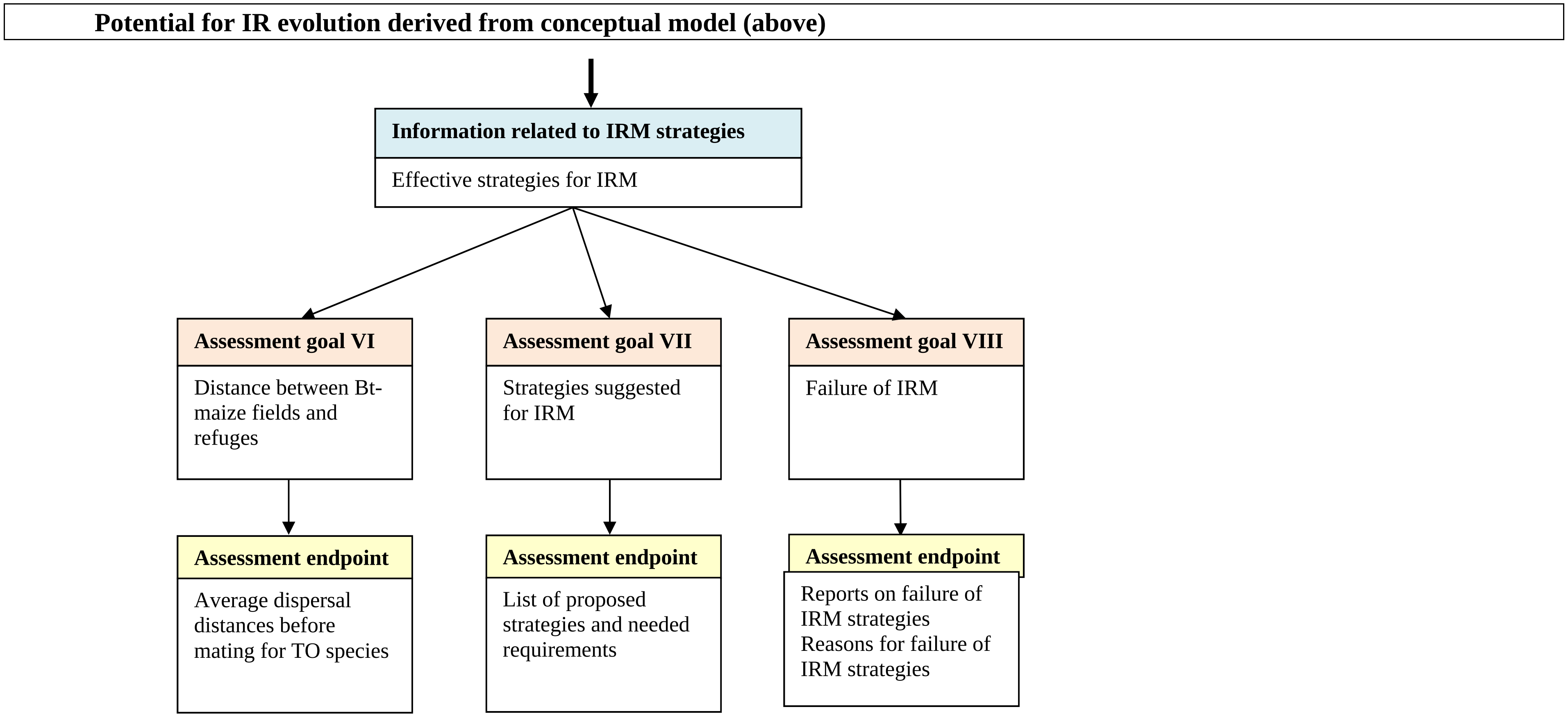


Figure 7: Conceptual model defining the scope for determining resistance evolution in target pests of Bt crops


Potential for IR evolution derived from conceptual model (above)
Information related to IRM strategies
Effective strategies for IRM
Assessment goal VI
Distance between Bt-maize fields and refuges
Assessment goal VII
Strategies suggested for IRM
Assessment goal VIII
Failure of IRM
Assessment endpoint
Average dispersal distances before mating for TO species
Assessment endpoint
List of proposed strategies and needed requirements
Assessment endpoint
Reports on failure of IRM strategies
Reasons for failure of IRM strategies

Specific Questions on Resistance Development and Resistance Management of target organisms exposed to specific Cry proteins

The questions on these topics do not always fit the **PICO** format and so the **PO (Population and Outcome conditions)** format is applied to these questions which will often result in qualitative reviews.

Scope of the question 1

The European corn borer *Ostrinia nubilalis* (Hübner) (ECB) and the Mediterranean corn borer *Sesamia nonagroides* (Lefèbvre) (MCB) are the key maize pests in Europe (Shelton et al. 2002) and major targets for transgenic *Bt*-maize expressing Cry 1 proteins (Fischhoff 1996). Furthermore the coleopteran maize pest *Diabrotica virgifera virgifera* LeConte has been repeatedly introduced to Europe since the 1990s (Miller et al 2005), which can be controlled by *Bt*-maize varieties expressing coleopteran specific Cry-proteins (Devos et al 2012). These species can cause severe economic damages in maize. However, other lepidopteran or coleopteran pests in maize may occur regionally and might be economically important pests (MacIntosh 2009). They will also have the potential to develop resistance to *Bt*-proteins expressed in GM maize. EFSA considers the evolution of resistance for these species as a concern, which should be assessed and observed (EFSA 2012). But little is known about these species which regionally occur in maize and cause economic damage.

Review question 1:

Which key and regionally occurring, economically important lepidopteran/coleopteran pests (P) occur on maize in Europe (O)?

Scope of review question 2:

One key assumption for the high-dose/refuge strategy is that the amount of *Bt*-protein expressed in *Bt*-maize is killing nearly all pest organisms feeding on GM plant (high dose). Therefore data on dose-response relationship is needed to estimate the baseline susceptibility of the target organism. Furthermore changes in the baseline susceptibility of the target organisms are measured in insect resistance monitoring programs indicating resistance evolution (Saeglitz et al 2006). The data on baseline susceptibility should enable risk assessors and managers to assess whether *Bt*-maize events represent a high-dose to a specific target organisms, resistance has developed after commercial introduction of Bt-maize events (monitoring) and to identify potential knowledge gaps.

Review question 2:

Are base line data available on the susceptibility to Bt-proteins (O) of different lepidopteran/coleopteran maize pests (identified in review question 1) (P)?

Scope of review question 3:

A further important factor to delay the evolution of resistance is the frequency of resistance alleles and their inheritance. The resistance alleles must be sufficiently rare, so that nearly all resistance alleles will be in heterozygotic genotypes. If nearly all resistance alleles will be in heterozygotes, they can be eliminated by the *Bt*-maize if it expresses high dose (Andow 2008). Resistance alleles are designated as rare, if their frequency is below 10^{-3} (Roush 1994).

Review question 3:

Is the frequency of resistance alleles lower than 10^{-3} (O) in lepidopteran/coleopteran maize pests (P) in different regions in Europe?

Scope of review question 4:

Models showed that most efficient delay in resistance evolution can be expected if the inheritance of resistance is completely recessive (Bates et al. 2005). Only in such cases heterozygous offspring resulting from crosses between resistant and susceptible individuals are expected to be susceptible to the *Bt*-toxin (Devos 2012).

Review question 4:

Is the inheritance of resistance alleles fully recessive (O) in populations of lepidopteran/coleopteran maize pest (P)?

Scope of review question 5:

The spread of resistance alleles might be affected by fitness costs, which are associated with resistance evolution. Fitness costs increase, if the resistant individual is less competitive to a susceptible one in the non *Bt*-maize. In consequence fitness costs would exert control over the frequency of resistance alleles, which results in a delay or reverse of resistance by selecting against resistant genotypes. Refuges would delay resistance evolution not only by providing susceptible individuals to mate with resistant individuals, but also by selecting against resistance (Gassmann et al. 2009, Devos et al. 2012).

Review question 5:

Is observed resistance of lepidopteran/coleopteran maize pests to *Bt*-crops (P) associated with fitness costs (O)?

Scope of review question 6:

The main tool for delaying resistance is the establishment of refuges. The refuges should be large enough to produce a sufficient number of susceptible individuals, which randomly mate with the rare resistant individuals emerging from the *Bt*-maize fields. These offspring are susceptible to the *Bt*-proteins, if the inheritance of resistance in recessive. Therefore knowledge on small scale migration of the relevant lepidopteran/coleopteran pest species before mating is essential to decide how far refuges can be located from Bt-maize fields to guarantee random mating (Showers et al. 2001).

Review question 6:

How far (O) do lepidopteran/coleopteran maize pest species (P) disperse before mating?

Scope of review question 7:
As outlined above the high dose refuge strategy is mostly recommended for delaying the resistance development. However, this strategy will only work, if the underlying assumptions are met (Tabashnik 1994, Tabashnik et al 2009, Tabashnik & Gould 2012). But this might not be the case for all relevant lepidopteran/coleopteran pest species. In consequence other strategies than the adoption of the high dose strategies are needed. Therefore it might be helpful for decision makers to get an overview on further options to delay resistance evolution.

Review question 7:

Which strategies and additional measures exist to delay the evolution of resistance development (O) of lepidopteran/coleopteran pests (P)?

Scope of review question 8:
GM crops expressing *Bt*-proteins to control lepidopteran/coleopteran pests were cultivated outside Europe for many years, and insect resistance management (IRM) strategies to delay resistance evolution were introduced concurrently. However, experience showed that the IRM failed in some cases and lepidopteran or coleopteran pests evolved resistance (Tabashnik et al 2009). Therefore it might be wise to collect and analyse data, which might help to improve and adapt IRM on European conditions.

Review question 8:

What reasons are described for the failure of insect resistance management strategies (O) for lepidopteran/coleopteran pests in *Bt*-maize (P)?

3.3.2 List of review questions on impacts of Bt crops on Target species

Review questions	
1	Which key and regionally occurring, economically important lepidopteran/coleopteran pests (P) occur on maize in Europe (O)?
2	Are baseline susceptibility data available on the Bt-proteins (O) of different lepidopteran/coleopteran maize pests (identified in review question 1) (P)?
3	Is the frequency of resistance alleles lower than 10^{-3} (O) in lepidopteran/coleopteran maize pests (P) in different regions in Europe?
4	Is the inheritance of resistance alleles (O) in populations of lepidopteran/coleopteran maize pest (P) fully recessive?
5	Is observed resistance of lepidopteran/coleopteran maize pests to *Bt*-crops (P) associated with fitness costs (O)?

6	How far (O) do lepidopteran/coleopteran maize pest species (P) disperse before mating?
7	Which strategies and additional measures exist to delay the evolution of resistance development (O) of lepidopteran/coleopteran pests (P)?
8	Which reasons are described for the failure of insect resistance management strategies (O) for lepidopteran/coleopteran pests in *Bt*-maize (P)?

3.4 CRY toxins: mode of action and risk assessment

CRY toxins are a unique group of proteins produced as extra-sporal crystals (CRYstals) by bacteria of the species *Bacillus thuringiensis.* A nomenclature based on the relatedness of the amino acid sequences of these proteins has been established. The knowledge about the biology of *B. thuringiensis* and the mode of action of a number of these toxins at the molecular level has increased significantly during the last ten years. However a number of issues need to be considered in relation to the risk assessment of CRY toxins produced by Bt plants. Reviews will be conducted which consider:

1. Does the knowledge about the biology of *B. thuringiensis* and its action towards target organisms (P) raise any new questions (I) in relation to the risk assessment of CRY toxins produced by GM plants (T)?
2. Does the knowledge about the mode of action of CRY toxins at the molecular level (P) pose any issues for the risk assessment of CRY toxins produced by GMPs (O)?
3. Is the nomenclature of CRY toxins derived from *B thuringiensis* (P) applicable (I) to the CRY toxins produced by plant (T)?
4. How closely should the nomenclature (I) be related in order for it to be relevant for the risk assessments of the proteins of different origin (P) to be comparable (T)?
5. Is it possible to connect the relatedness of CRY toxins (P) with their activity (O) towards arthropods – even at the level of insect orders?
6. What current knowledge is there to conclude on whether the effects of combined, stacked or pyramided CRY toxins (P) , as produced by stacked GMPs, will be additive, synergistic or antagonistic (O)?

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