Synthesis and Overview Studies to Evaluate Existing Research and Knowledge on Biological Issues on GM Plants of Relevance to Swiss Environments

Report

Author(s):
Sweet, Jeremy; Bartsch, Detlef

Publication date:
2012

Permanent link:
https://doi.org/10.3929/ethz-a-007350187

FNSNF
Swiss National Science Foundation

Jeremy Sweet
Detlef Bartsch

National Research Programme NRP 59 “Benefits and Risks of the Deliberate Release of Genetically Modified Plants”: Review of International Literature

Synthesis and Overview Studies to Evaluate Existing Research and Knowledge on Biological Issues on GM Plants of Relevance to Swiss Environments

vdf

National Research Programme NRP 59 “Benefits and Risks of the Deliberate Release of Genetically Modified Plants”: Review of International Literature

Synthesis and Overview Studies to Evaluate Existing Research and Knowledge on Biological Issues on GM Plants of Relevance to Swiss Environments

Dr. Jeremy Sweet
Member of the NRP 59 Steering Committee
Environmental and Research Consultant,
Cambridge (UK)

Prof. Dr. Detlef Bartsch
Member of the NRP 59 Steering Committee
Federal Office of Consumer Protection and Food
Safety, Berlin (DE)

vdf Hochschulverlag AG an der ETH Zürich

Published with the support of the
Swiss National Science Foundation.

Bibliographic Information published by Die Deutsche Nationalbibliothek
Die Deutsche Nationalbibliothek lists this publication in the Internet at http://dnb.d-nb.de.

ISBN 978-3-7281-3498-1 (Print version)

ISBN 978-3-7281-3499-8 / DOI 10.3218/3499-8

www.vdf.ethz.ch, verlag@vdf.ethz.ch

Correspondence: Jeremy Sweet: Jeremysweet303@aol.com
Website: www.nfp59.ch

Aim of the three literature reviews

The National Research Programme "Benefits and Risks of the Deliberate Release of Genetically Modified Plants (NRP 59)" consists of four main areas of interest:

1. Plant biotechnology and the environment
2. Social, economic and political aspects
3. Risk-assessment, risk-management and decision-making processes
4. Synthesis and overview studies

It was neither in the capacity nor in the scope of NRP 59 to duplicate the many studies on benefits and risks associated with genetically modified plants (GMP) that have been carried out in other parts of the world. On the other hand, it may be possible to distil relevant and valuable scientific data from the results of such studies that could help to shape future research and decision-making processes specifically tailored for Switzerland. In the frame of focus point IV, three overview studies were therefore compiled by members of the Steering Committeee of NRP 59 that evaluate on an international scale existing research and knowledge on topics that are of direct relevance to the central themes of NRP 59.

In the volume "*Medical issues related to genetically modified plants of relevance to Switzerland"* Karin Hoffmann-Sommergruber and Karoline Dorsch-Häsler provide an extensive overview of health-related risks and benefits of GM plants.

In the volume *"Genetically modified crop production: social sciences, agricultural economics, and costs and benefits of coexistence",* Joachim Scholderer and Wim Verbeke assembled valuable insight obtained by screening literature databases and research/project portals, and through direct contacts with key researchers in the different areas.

In a comprehensive third volume entitled *"Synthesis and overview studies to evaluate existing research and knowledge on biological issues on GM plants of relevance to Swiss environments"*, Jeremy Sweet and Detlef Bartsch compiled information resulting from close to one thousand scientific publications relating to biological and environmental issues on GMP.

The chapters in this volume will not only be useful to a readership that is familiar with the biological, environmental, political, socio- and agro-economical aspects of GMP, it will also provide newcomers to the field with an in-depth introduction into a range of specialised topics that are relevant to this complex area.

Table of Contents

1. Introduction

This study is an assessment of the extent that worldwide scientific information on GMOs is relevant to Switzerland. The results of this study will feed into the final report to be submitted to the Federal authorities in 2012.

An environmental risk assessment for GM plants is required by regulatory agencies worldwide. However, there are considerable differencies regarding assessment concepts, data requirements, their analysis and interpretation (e.g. EFSA, 2010a, Graef et al., 2010, Gressel, 2010, Herman, 2010, Raybould, 2010, Schubert, 2010, Hilbeck et al., 2011). Little attention is given to cost-benefit assessments and the EU regulatory approach is specifically focused on risks rather than benefits of GMO (EFSA, 2008d, Miller and Bradford, 2010, Tabashnik, 2010), which is probably one of the reasons why there is an asynchronus authorization of GM plants worldwide. However, trends of globalisation and worldwide trade (see e.g. Anderson, 2010, Beddington, 2010) have a number of implications for the assessement and regulation of GM plants. In addition, progress in plant breeding and genetic engineering techniques put constant challenges to current risk assessment approaches and the sustainable use of GM plants (e.g. COGEM, 2010, Cominelli and Tonelli, 2010, Fedoroff, 2010, Fedoroff et al., 2010, Jaggard et al., 2010, Llorente et al., 2010, Piesse and Thirtle, 2010, Franke et al., 2011, Hunter, 2011).

This NFP59 Module 4 study analyses and compiles the current state of knowledge on the environmental impacts of GM plants and crop coexistence from international research results. This report is a Synthesis Document encompassing a summary of the existing knowledge based on the evaluation of international research, complemented with findings obtained to date. Today, more than 11,000 studies about safety and coexistence aspects of GMO (including GM plants) have been published and made publically available with their abstracts in databases (See Figure 1).

About 1000 relevant scientific publications including a number of synthesis, review, and meta-analysis studies about GM plants and their potential effects on the environment and on coexistence were analysed in detail in this report (for methodology see chapter 2).

The report incorporates views on the implications for Switzerland of current research knowledge and addresses the principle issues and questions raised by a range of stakeholders. The report contributes towards the future debate on the commercialisation of GMOs in Switzerland.

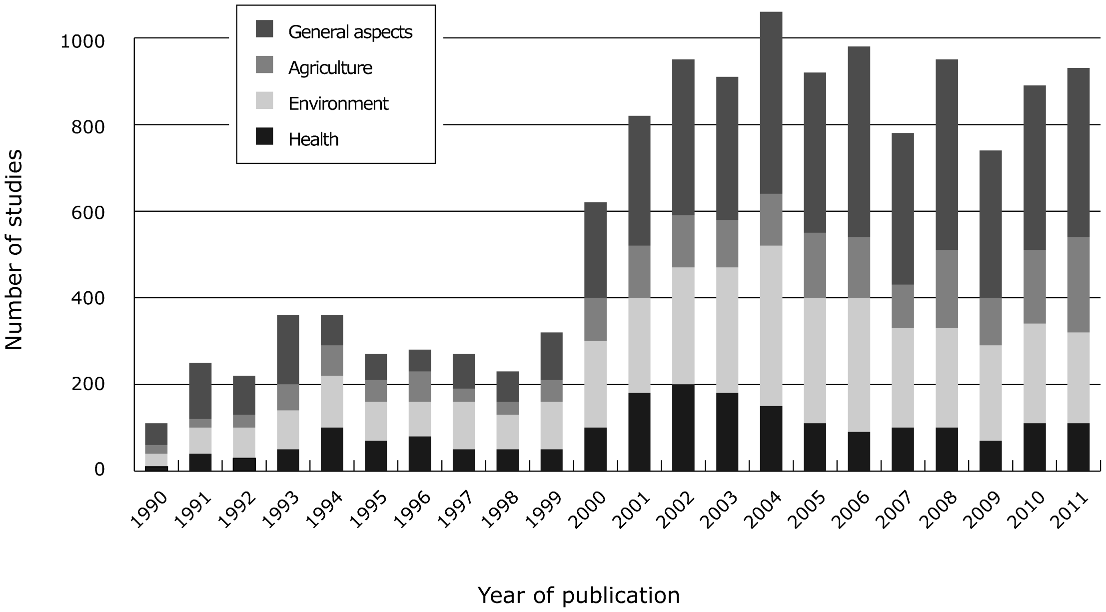


Figure 1: Publically available studies on biosafety and coexistence aspects of GMO with publication date since 1990 divided into for sections: General aspects, Agriculture, Environment, and Health. Source ICGEB Biosafety Bibliographic Database including 11,663 studies available on 6 June, 2012. Potential adverse effects of GMOs have been identified in many publications, but only a few of these effects have been confirmed in laboratory or greenhouse studies. No field scale adverse effects have been detected associated with cultivation of approved GM plants in the EU, though field experimental studies have shown the potential for Bt and HT crops to have some adverse effects (see chapter 4 for details).

2. Methodology

In the early 1990s the first large, publicly financed biosafety research programmes started in the EU as well as in many other countries worldwide. These research programmes accompanied the engineering of GM plants, for which one of the fathers of GM plants – Marc van Montagu – recently gave a very personal but nevertheless remarkable overview (Van Montagu, 2011).

An international community of researchers studying biosafety was developed and the International Society for Biosafety Research (ISBR) was formulated. National and international meetings such as the biennial International Symposium on Biosafety of GMOs organised by ISBR commenced. The focus of much of the scientific activity was to inform and elaborate the science underpinning the risk assessment and risk management of GMOs. The regulation, risk assessment and risk management of GMOs worldwide is largely based on this science, though socio-economic and political factors have considerable influence on regulatory and management practices. This study collected and collated information on identified and potential effects of GM crops on agriculture, the environment and on coexistence between GM and non-GM systems. The data are analysed to highlight and summarise the most important information and then this summary is related to Swiss agriculture, environment and political issues.

2.1 Sources of Biosafety Information

A broad spectrum of sources of information was studied, such as internet based information, library catalogues (International Centre for Genetic Engineering and Biotechnology (ICGEB)), online literature databases (e.g. ISI Web of Science, Medline), and conferences reports (e.g. Sweet, 2009). Additionally unpublished data (e.g. reports from premarketing experiments, personal communications with experts from science and regulation) were analysed. In particular resources in countries with a long experience of GMO cultivation (in particular USA, Canada, (see NRC, 2010) but also Argentina, Brasil and Australia) were explored as well as resources from countries with considerable biosafety research programmes. The findings of recent major European studies such as AIGM (ESF project), Ecogen and SIGMEA (EC projects), BEETLE (2009), and a review of EU funded GMO research (EU, 2010) are summarised. In addition Biosafenet (a network of European research scientists on biosafety) were exploited. This literature survey continues in major parts the BEETLE (2009) structure and findings.

The range of GM plant (GMP) species studied includes the majority of GMPs that could be potentially grown or introduced (intentionally or unintentionally) into Switzerland, including maize, oilseed rape, sugar beet, soya, potato, sunflower, cereal crops (wheat, barley etc.), fruit crops (apple, plum, strawberry, melon etc), flowers (carnation, chrysanthemum etc.) herbage and forage crops (e.g. alfalfa, grasses) vegetables (e.g. tomato, pepper, aubergine, Brassicae, etc.).

2.2 Legal Framework of ERA in the EU

In the EU, the environmental risk assessment must be carried out in accordance with the objectives, elements, principles and methodology laid down in Annex II to Directive 2001/18/EC. This Annex has been supplemented by guidance notes established in Commission Decision 2002/623/EC of 24 July 2002.

The objective of the environmental risk assessment is, on a case by case basis, to identify and evaluate potential direct or indirect, immediate or delayed adverse effects of the deliberate release of the GMO, on human and animal health and the environment. The Module 4 literature survey analysed the most relevant information from various sources and grouped the knowledge according to the main points as listed in Directive 2001/18/EC (section D2 of Annex II):

1. Likelihood of the Genetically Plant (GMP) becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.

2. Any fitness advantage or disadvantage conferred to the GMP.

3. Potential for gene transfer to the same or other sexually compatible plant species and any fitness advantage or disadvantage conferred to those plant species.

4. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMP with target and non-target organisms, such as predators, parasitoids, and pathogens (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.

5. Possible immediate and/or delayed effects on human and animal health resulting from potential direct and indirect exposure to the GMP.

6. Possible immediate and/or delayed effects on biogeochemical processes resulting from direct and indirect interactions of the GMP with soil functional systems.

7. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMP where these are different from those used for non-GMPs.

All collected data were assessed and conclusions compared with results generated in the relevant NFP59 projects on impacts on the environment. The potential benefits of GMPs are also assessed particularly in relation to benefits for central European agriculture and the environment.

Within the EU, EFSA is responsible for the scientific assessment of the release applications for GMOs and their associated risk assessments, and gives scientific opinions on the safety of GMOs for human and animal health and the environment. This includes providing independent scientific advice on the safety of:

- genetically modified organisms (GMOs) such as plants, animals and micro-organisms, on the basis of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, including genetically modified plants

- genetically modified food and feed, on the basis of Regulation (EC) No 1829/2003 on genetically modified food and feed

The EFSA GMO Panel assesses the risks of all GMO applications in order to produce scientific opinions and advice for the EC and members state risk managers. Its risk assessment work is based on reviewing scientific information and data in order to evaluate the safety of a given GMO. This helps to provide a sound foundation for European policies and legislation and supports risk managers in taking effective and timely decisions. European legislation requires that an applicant must submit an application for any GMO to be authorised in the EU for placing on the market. This application must contain a risk assessment conducted according to the requirements of the EU regulations. In accordance with EU legislation, an independent scientific assessment is carried out by the EFSA's GMO Panel to evaluate the risk assessment of the applicant and give an opinion on safety of the GMO. The Panel's independent scientific advice is then used by the Commission and Member States when taking a decision on market approval. As part of its remit, the EFSA GMO Panel produces a number of Guidance Documents to provide the applicants with guidance for the preparation and presentation of applications and to clarify its approach to risk assessment and to ensure transparency in its work. Recently, a new EFSA publication (EFSA, 2010a) provided more detailed guidance for the environmental risk assessment (ERA) of genetically modified (GM) plants submitted within the framework of Regulation (EC) No 1829/2003 on GM food and feed or under Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (GMOs).

The EFSA ERA guidance document (EFSA, 2010a) was subject to intensive discussions and consultations with a wide range of stakeholders and several alternative approaches were considered during the exercise. Recent critical reviews of risk assessment methodologies (e.g. by Myhr, 2010, Hilbeck et al., 2011 or Meyer, 2011) have indirectly confirmed the scientific robustness and applicability of the EFSA approach.

Risk assessment serves as the scientific basis for informing decision making as laid down in national and international regulations (see Sparrow, 2010). Risk assessment is subject to considerable scientific, political, and public scrutiny (Abt et al., 2010). With increasing complexity and demands, the risk assessment process is challenged to provide decision-relevant information in a timely fashion, with some assessments in the GM Agriculture area taking more than 10 years to complete. According to Davison (2010) the EU has the probably strictest regulations in the world for the presence of GMOs in food and feed, which is likely to be true for both risk assessment and coexistence issues.

The ERA of GM plants involves generating, collecting and assessing information on a GM plant in order to determine its impact on human/animal health and the environment relative to non-GMOs, and thus assessing its relative safety. The EFSA ERA document provides guidance to risk assessors for assessing potential effects of GM plants into the environment and the rationales for data requirements in order to complete a comprehensive ERA, and to draw conclusions for the post-market environmental monitoring (PMEM). The ERA should be carried out in a scientifically sound manner based on available scientific and technical data and on common methodology for the identification, gathering and interpretation of the relevant data. Tests, and measurements, and data generated should be clearly described as well as the assumptions made during the ERA. In addition, the use of scientifically sound modelling approaches could provide further useful information for the ERA. Sufficient scientific data must be available in order to arrive at qualitative/quantitative risk estimates.

Each risk assessment begins with problem formulation in which the most important questions that merit detailed risk characterisation are identified (see chapter 3.1). Problem formulation helps to make the risk assessment process transparent by explicitly stating the assumptions underlying the risk assessment. At the end, the overall risk evaluation should result in informed qualitative and, if possible, quantitative advice to risk managers, outlining the nature and magnitude of uncertainties associated with the identified risks (see chapter 5.1). The implications of the risk assessment for risk management measures should also be assessed.

The EFSA ERA document considers that seven specific areas of concern should be addressed by applicants and risk assessors during the ERA (1) persistence and invasiveness of the GM plant, or its compatible relatives, including plant-to-plant gene transfer; (2) plant-to-micro-organism gene transfer; (3) interaction of the GM plant with target organisms; (4) interaction of the GM plant with non-target organisms, including criteria for selection of appropriate species and relevant functional groups for risk assessment; (5) impact of the specific cultivation, management and harvesting techniques; including consideration of the production systems and the receiving environment(s); (6) effects on biogeochemical processes; and (7) effects on human and animal health. Each specific area of concern is considered in a structured and systematic way following the above-mentioned steps (1 to 6). In chapter 4 of this NFP59 Module 4 report the main scientific and biosafety issues associated with these seven areas of concern are addressed.

The ERA should follow a weight-of-evidence approach considering intended and unintended effects.

The ERA should be carried out on a case-by-case basis, meaning that the required information may vary depending on the type of the GM plants and trait(s) concerned, their intended use(s), and the potential receiving environment(s). Information for ERA can be collected via (1) field-generated data (from field trials, field surveys, semi-field trials, and/or agronomic field trials), (2) molecular characterisation data, (3) compositional data, (4) laboratory studies (including ecotoxicological studies , (5) modelling, and/or (6) desk and literature studies.

In addition, the EFSA Guidance Document (EFSA, 2010a) is supplemented with several general cross-cutting considerations (e.g. choice of comparator, receiving environment(s), general statistical principles, long-term effects) that need to be considered in the ERA. The scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) proposes a step-wise selection process of relevant receiving environments to be addressed for ERA of a GM plant in question. Applicants should follow general statistical principles as outlined in this document. If experimental studies are being used they should allow testing for difference and equivalence. The EFSA GMO Panel also provides statistical guidance for specification of effect size, limits of concern, power analysis, experimental design, analysis and reporting. Recommendations are given how to address uncertainty (see also Van der Voet et al., 2011).

Predicting impacts of GM plants on complex ecosystems which are continually in flux is difficult and largely based on experiences with other introductions and an understanding of the robustness of ecosystems. It is recognised that an environmental risk assessment is limited by the nature, scale and location of experimental releases, which biospheres have been studied and the length of time the studies were conducted.

The assessment of long-term effects requires specific information sources and techniques, including experimental or theoretical methodologies, and recommendations for establishing relevant baseline information. Scientific knowledge and experience gained from growing GM plants during the monitoring of experimental releases and provisional approval periods for GM plants will also inform the risk assessment process and are opportunities to continually update environmental risk assessments in the light of any new knowledge.

The risk characterisation should include considerations:

- whether cultivation of GM plants is as safe for the environment as the cultivation of non-GM plants;

- whether consumption of foods/feed derived from GM plants is as safe for humans/animals as the conventional comparators;

- whether specific conditions for GM plant cultivation, may be required;

- regarding the scientific basis for different options to be considered for risk management, including post market environmental monitoring.

Where GM events have been approved under Regulation (EC) No 1829/2003 or Directive 2001/18/EC, genotypes produced by crossing plants containing these events with non-GM plants are not required to undergo further risk assessment. However, where applications involve the crossing of GM plants to stack GM events, a risk assessment is required in the European Union, even when the single events comprising the stack have been approved. This regulation differs from that operating in some other countries (e.g. USA). The stacking of approved events can arise from unintentional crosses as has happened in Canada with both oilseed rape and its weedy relative *Brassica rapa* (e.g. Knispel et al., 2008, Londo et al., 2010) and has occurred in rice and wheat breeding programmes in USA with unapproved events (EC 2006, EFSA, 2006, Gaines et al., 2007). Stacked events have become more important in recent years. The first cultivation started in 1997-1999 with a stacked event of insect resistance (IR) and herbicide tolerance (HT) in cotton and maize. Today, the most common stacked events are combinations of (i) different IR genes or (ii) of IR and HT genes, obtained by the crossing of single (or double trait ie IR +HT) paternal lines. In some cases the HT genes are present more as selective markers than for allowing use of the specific herbicide. An increasing number of stacked events are now being submitted for both food and feed importation and for cultivation in the EU (EFSA, 2007a; De Schrijver et al, 2007). Based on the EFSA Guidance Document (EFSA, 2010a) the ERA should take into account the evaluation of the individual events and additional data from molecular characterisation and comparative compositional analysis of the stacked events when determining potential interactions between genes or between gene products. In line with the BEETLE report (2009) and the US National Research Council (NRC 2010) this Module 4 report confirms that no detrimental negative interaction has been observed between stacked genes in GM crops so far. In addition, the EFSA GMO Panel recently dismissed claims of adverse interactions thought to be inherent to stacked events by an NGO organisation (EFSA, 2011a). Another issue to be considered is that stacked events naturally segregate in the process of reproduction, and recent scientific opinions of EFSA regarding stacked events therefore consider all sub-combinations independently of their origin (e.g. EFSA, 2011e).

2.3 Legal Framework of Coexistence in Europe

Coexistence of GM crops and products with non GM crops and products is primarily a socio-economic issue associated with supplying foods and feeds of known GM or non-GM purity. In order to achieve certain thresholds of purity it is necessary to segregate agricultural production systems and supply chains. Scientific knowledge is required to understand the sources of cross contamination and to manage the levels within required thresholds.

The important routes for cross contamination in agriculture are through cross pollination and seed dispersal, both natural and man induced. Considerable research has been conducted on the nature of pollen dispersal, the biology of cross pollination and the genetics of seed set (see review by Eastham and Sweet, 2002). In addition studies have been conducted on a range of physical and biological methods to minimise or restrict pollination and seed set. There is also considerable research information on seed dispersal, dormancy, longevity and viablility in the major crop species. This research was reviewed and put into the context of studies in Switzerland where there has been considerable research on managing coexistence of GM and non GM maize and some studies of other crops (see chapter 6).

Specifically information was obtained from the six international coexistence conferences (GMCC03[1], GMCC05[2], GMCC07[3], GMCC09[4], GMCC11[5] and SGFA11[6]), from international studies and from the EU projects SIGMEA, Co-Extra and Transcontainer. In addition the co-existence and gene flow studies conducted in Switzerland were examined. The issues of GM crops with stacked events are also considered within coexistence measures (Paul et al., 2011).

2.4 GM Plants of Interest for Switzerland

Present and future GM plants that are cultivated or might be considered for cultivation in Europe are among others:

- Maize
- Soybean
- Oilseed rape and other oil crops (eg sunflower)
- Cotton
- Rice
- Beet
- Potato
- Wheat and other cereals
- Vegetables
- Fruit crops
- Trees
- Grass and forage crops

---

[1] GMCC03: First European Conference on Co-existence of Genetically Modified Crops with Conventional and Organic Crops. http://www.agrsci.dk/gmcc-03/

[2] GMCC05: Second International Conference on Co-existence between GM and Non-GM based agricultural supply chains. http://www.gmcc05.com/

[3] GMCC07: Third International Conference on Co-existence between GM and Non-GM based agricultural supply chains. http://gmcc-07.jrc.ec.europa.eu/news.htm

[4] GMCC09: Fourth International Conference on Co-existence between GM and Non-GM based agricultural supply chains. www.gmcc-09.com/

[5] GMCC11: Fithth International Conference on Co-existence between GM and Non-GM based agricultural supply chains. http://gmcc-11.com/

[6] SGFA11: The Science of Gene Flow in Agriculture and its role in co-existence. Conference UC Davis. http://sbc.ucdavis.edu/files2/geneflowcompleteproceedings2011.pdf

Some of these crops (like cotton, rice) can only realistically be cultivated in some warmer regions, while others, like potato, may (potentially) be cultivated throughout Europe. Therefore in risk assessment the existing geographical zones should be coupled to the likely cultivation area of specific GM crops, considering the crop*trait combination.

The potential environmental risk associated with some of these crops is discussed in the following Sections, considering particularly crops of interest to Switzerland: wheat, potato, maize, sugar beet, oilseed rape, apple, and grape vine (BATS, 1996).

3. Environmental Risk Assessment

The risk assessment strategy for GM plants seeks to use appropriate methods to compare the GM plant and derived products with their appropriate comparator. Thus non-GM plants serve as comparators for the ERA of GM plants. The comparative safety assessment is being followed in order to identify differences caused by either intended or unintended effects of the transformation process and the expression of the novel gene construct. This 'Comparative Safety Assessment' is still subject to intensive discussion (Abt et al., 2010, Kuiper and Davies, 2010, Herman, 2010, Hilbeck et al., 2011, Meyer, 2011, Parrott et al., 2010, Rüdelsheim and Smets, 2010).

Comparative safety assessment includes molecular characterisation, the agronomic and phenotypic characteristics of the GM plant in question, as well as its compositional analysis (OECD, 1993, FAO/WHO, 1996). In addition, the comparative safety assessment within ERA shall use information on the interactions of the GM plant with its receiving environment(s) in comparison with those associated with similar conventional plants.

Genetic modification of plants results in the intended effects required by the plant breeder, but may also result in unintended effects. There is increasing evidence that 'classical breeding' technologies such as mutation breeding, radiation breeding, and other technologies including chemical treedments may cause more physiological and phenological alterations than genetic engineering (Arber 2010, Barros et al., 2010, Coll et al., 2010a, 2010b, Harrigan et al., 2010, Kogel et al., 2010, Herman et al., 2011).

The ERA is focused on the identification and characterisation of both effects with respect to possible adverse impacts on human and animal health and the environment. Effects can be direct and indirect, immediate and delayed, including cumulative long-term effects.

Intended effects are those that are designed to occur and which fulfil the original objectives of the genetic modification. Alterations in the phenotype may be identified through a comparative analysis of growth performance, yield, pest and disease resistance, etc. Intended alterations in the composition of a GM plant compared to its appropriate comparator, may be identified by measurements of single compounds.

Unintended effects of the genetic modification are considered to be consistent (non-transient) differences between the GM plant and its appropriate comparator, which go beyond the primary intended effect(s) of introducing the transgene(s). Since these unintended effects are event-specific, applicants must supply data on the specific event. In general, it can be concluded that very few clearly unexpected effects were observed during the large scale post-release growing of herbicide-tolerant crops and Bt crops within the last 10 to 15 years (Van den Brink et al., 2010). One has to keep in mind that there will always be an element of subjectivity in assessing effects as "unexpected". Furthermore, it is also possible that certain effects are becoming visible only after a longer period than 10 to 15 years, but no indications for such effects were found in the literature (see e.g. Van den Brink et al., 2010, BEETLE, 2010). Unexpected effects caused directly by the genetic modification were not found. For example, Himanen et al. (2010) tested whether Bt transgenes may have unintended effects: The presence of transgenes did not perturb fecundity, within-plant biomass allocation or O_3 tolerance of *B. napus.* Only in herbicide-tolerant crops, Brink et al. (2010) concluded to some indirect unexpected effects: the reduced uptake of micro-nutrients and some positive and negative effects on susceptibility to diseases. These effects are specific to herbicide use with glyphosate-tolerant GM and non-GM crops.

The sources of data that are used to determine unintended effects are:

1. Molecular characterisation: A starting point in the identification of potential unintended effects is analysis of the DNA construct and insertion site to establish whether the insertion is likely to have potential effects other than the intent of the original genetic modification (e.g. unintended effect(s) could be due to loss of function of an endogenous gene at the insertion site). However, there seems to be high genetic stability in commercialized GM plants to date (e.g. for Bt maize: Ben Taher et al., 2010).

2. Compositional analysis: Unintended effects may be detected through the comparison of the compositional characteristics of the GM plant with its appropriate comparator (e.g. unintended effect(s) could potentially be linked to metabolic perturbations).

3. Agronomic and phenotypic characterisation: Unintended effects may also be detected through the comparison of the phenotypic and agronomic characteristics of the GM plant with its appropriate comparator (e.g. unintended effects could be linked to morphological alterations).

4. GM plant-environment interactions: Unintended effects may be detected through comparisons of biotic and abiotic interactions of the GM plant and its appropriate comparator with components of their receiving environment(s). *In planta* data are the fundamental source of information (e.g. unintended effects could be linked to changes in the interaction of the GM plant on functionality of NTO guilds).

For ERA, statistically significant differences between the GM plant and its appropriate comparators, which are not due to the intended modification, may indicate the occurrence of unintended effects, and should be assessed specifically with respect to their biological relevance and potentially hazardous environmental implications. The outcome of the comparative safety assessment allows the determination of those “identified” characteristics that need to be assessed for their potential adverse effects in the environment, regardless of whether they were intended or unintended, and will thus further structure the ERA.

Raybould et al. (2011) studied how laboratory methods for assessing the ecotoxicological effects of synthetic pesticides have been modified for the measurement of effects of insecticidal proteins, and how these effect measures are combined with exposure estimates to derive ‘Hazard Quotients’ for assessing the ecological risks from the cultivation of insect-resistant transgenic crops. Allthough the potential for such ecological modeling to inform the design of laboratory effects tests for insecticidal proteins is discussed by Raybould et al. (2011) it remains unclear how this approach might improve the ERA in the future.

3.1 Problem Formulation: Context and Definitions

Problem formulation is the critical first step of the environmental risk assessment (ERA). It extracts policy goals, scope, assessment endpoints and methodology to an explicitly stated problem and approach for analysis (Figure 2). Improving the consistency and value of ERAs conducted for genetically modified (GM) plants can be achieved through rigorous problem formulation (PF) whereby the problem context for risk assessment is transformed through problem definition to an analytical plan that describes relevant exposure scenarios and the potential consequences of these scenarios (Wolt et al., 2009). Formal definition of the ERA through a properly executed PF assures the relevance of risk assessment outcomes for decision-making. Adopting consistent problem formulation will bring greater uniformity in ERAs among various regulatory regimes.

The PF should also explicitly state significant assumptions underlying the risk assessment. For example, even the term ‘environment’ requires operational definition in a given ERA. This is because, depending on the specific problem context, the ERA may encompass consideration of anthropogenic as well as natural components; and human and animal health needs to be considered according to Directive 2001/18/EC.

In terms of assessment endpoints, there needs to be a common understanding of adverse effects for hazard and value identification (see chapter 3.2). One crucial point is whether PF should consider the occurrence of pleiotropic effects, for which it is per se hard to give any case-specific prognosis. Pleiotropic and other unanticipated effects may be important to assess for certain types of GM plants and they should be taken into consideration when formulating the problem and developing risk hypotheses. Considering pleiotropic effects may call for more data and this might increase rather than allay concerns about the environmental impacts of GMOs (Raybould 2006, Johnson et al., 2007).

The PF needs to include specified or appropriate assessment endpoints, and sufficient clarity on the purpose and use of information requested by risk assessors and decision-makers.

Based on the EFSA ERA guidance document (EFSA, 2010a) the ERA is conducted starting with step 1 and moving towards step 6; step 2 and 3 can, however, be carried out in parallel (see Figure 2). The EFSA document provides detailed guidance on how to consider appropriate comparators, long-term effects and general aspects of statistical data analysis.

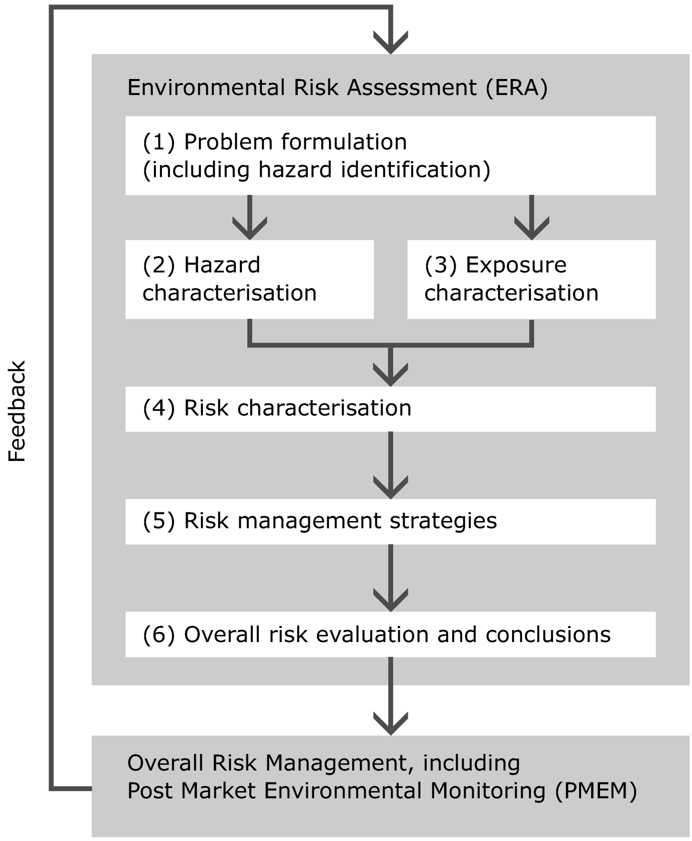


Figure 2: Six steps within the environmental risk assessment (ERA) and relationship to risk management, including monitoring, according to Directive 2001/18/EC and Regulation (EC) No. 1829/2003, as outlined by EFSA (2010a).

3.2 Environmental Protection Goals

Environmental Protection Goals (EPG) need to be defined according to national (Swiss) and internationally binding legislation. For the EU, some EPGs are defined in the Fauna-Flora-Habitat Directive (see Table 1), such as biodiversity, species protection, habitat conservation etc. The aim of this Directive is to contribute towards ensuring biodiversity through the conservation of natural habitats and of wild fauna and flora in the European territory of the Member States to which the Treaty applies. This comprises not only protected species and habitats, but also ecosystem functions, such as pollination or biological control, protection of soil functions, quality of water and air. Related to this, sustainable agriculture is also described as an EPG since it is generally considered to be an approach for conserving Biodiversity in agro-ecosystems which are heavily influenced by man. Recent alternative approaches to define protection goals considering effects of GMO are available from Bartz et al. (2009), Heink et al. (2010), and NABU (2010). Considerable work on technology assessment and protection goals for Switzerland was already carried out during the BATS research project 'Nachhaltige Landwirtschaft' (BATS 2000).

Biodiversity is an important element in determining protection goals and biodiversity covers both species richness and ecological services (including agro-eco-functions). Both components are clearly linked; for example rareness and conservation/protection status are often linked. However ecosystem function may not always be dependent on the number of species but the ecological niche(s) they occupy and their role in those niches (see Finke and Snyder, 2008).

In relation to environmental impact assessment of any developments on (wider) biodiversity, most European countries have put into place assessment procedures to identify projects of high risk to biodiversity, in line with Article 14 of the CBD[7]. The EU legislation is available at http://europa.eu.int/comm/environment/eia/home.htm.

All agriculture, forestry, and landscape management systems have impacts on biodiversity. For example, *Bacillus thuringiensis israelensis* (Bti) is the most commonly used – conventional – microbial pesticide to control mosquitoes worldwide, including sensitive areas like the Camargue in France. It has been shown by Poulin et al. (2010) that there can be negative effects on breeding bird populations. Thus mosquito-control programmes should integrate non-biased awareness campaigns and mitigation measures balancing the social demands for mosquito reduction with the factors involved in mosquito proliferation and dispersion. Such measures could consist in improved agricultural and wetland management; reduction in areas and periods of Bti spraying; consideration of alternatives to Bti spraying, such as mosquito traps; specific measures to reinforce animal populations affected by Bti; and suspension of mosquito control in environmentally sensitive areas where nature preservation is a priority. It is well documented that changes in the scale of farms, farming practices and the use of pesticides has resulted in declines in farmland biodiversity in many parts of Europe and elsewhere (Donald et al., 2006, Reidsma et al., 2006, Reif et al., 2008, Hawes et al., 2010, Potts et al., 2011). Natura 2000 and the agro-environmental schemes developed in many countries are trying to arrest this decline and improve biodiversity in some regions. In addition moves towards integrated pest management as a component of more sustainable farming systems are making farmers and land managers aware that biodiversity can be exploited in order to protect and enhance crop performance. Thus managing functional biodiversity should be an important component of farm management systems. It is against these farming systems that systems incorporating GM plants should be assessed and their risks and benefits to these systems should be considered.

---

[7] Convention on Biological Diversity: http://www.cbd.int/

It is important that the environmental risk assessment of GM crops identifies whether their impacts are different from those of comparable conventional cropping systems. To scientifically assess risks, it is necessary to test hypotheses and identify clear and objective protection goals around which assessment and measurement endpoints are developed for the environmental risk assessment of GM plants. The issue of selecting an ‘appropriate’ or ‘acceptable’ baseline level of biodiversity for any agro-ecosystem is widely debated. Logically, an ‘acceptable’ level of biodiversity needs to be determined and agreed. This acceptable level needs to be above a ‘minimum’ biodiversity level for the efficient and sustainable functioning of the particular agro-ecosystem (i.e. providing essential ‘biological services’, including biological control of pests and diseases, nutrient fixing and cycling, maintenance of soil moisture and structural stability, etc). Once an acceptable level of biodiversity is known for a particular agro-ecosystem, it should then be possible to design the management of the crop towards achieving or maintaining this desired level of biodiversity. The required level of biodiversity in a particular agricultural system is therefore considered by many to be a subjective or cultural response in an artificial (human-managed) environment, rather than a basic and definitive biological measure. Since agro-ecosystems are human-modified environments, it is logical to expect biodiversity levels to be different from those of natural habitats. Providing more bio-diverse semi-natural habitats in areas adjacent to farmed land can maintain biodiversity levels. These wildlife refugia are more important to provide when farming is more intensive, less fragemented and has little crop diversity, especially if integrated management systems are to be exploited (e.g. the ‘push-pull’ strategy for maize IPM, Cook et al., 2007). The spatial deployment of pest susceptible ‘refugia’ areas within pest-resistant GM crops illustrates that larger scale approaches can be devised and successfully deployed at a regional scale if there are sufficient incentives (contractual, financial, etc.). It is therefore important that the environmental risk assessment takes into account the possible threats to the biodiversity within the agro-ecosystems and in the surrounding habitats, particularly considering the possible implications for protected areas that might be in proximity of cropping areas.

Table 1: Examples of environmental protection goals and their legal bases in the EU. Directive 2001/18/EC (a) specifically applies to GM plants. Other legislations as listed below should be considered by the applicant.

Protection goals		Legal basis	
Areas of protection		Background	Scope
Biodiversity conservation	Species of conservation or cultural value; red list species // Protected habitats; landscapes	Directive 2004/35/EC(b)	Environmental liability
		Directive 92/43/EEC(c)	Conservation of natural habitats and of wild fauna and flora
		Directive 2009/147/EC(d)	Conservation of wild birds
		Regulation 338/97(e)	Protection of endangered wild fauna and flora
		Action plan for biodiversity(f)	Conservation of biodiversity
		Biodiversity strategy(g)	Conservation of biodiversity
		Biodiversity action plan for the conservation of natural resources(h)	Conservation of natural resources
		Biodiversity action plan for agriculture(i)	Conservation of biodiversity
		Bern convention(j)	Conservation of European wildlife and natural habitats
		Convention on biological diversity(k)	Conservation of biological diversity
Ecological functions	Soil	Directive 2004/35/EC	Environmental liability
		Thematic strategy for soil protection(l)	Preservation of soil functions
	Water	Directive 2000/60/EC(m)	Water protection
	Production systems; plant health	Regulation 1107/2009(n)	Marketing of plant protection products
		Directive 2009/128/EC(o)	Sustainable use of PPP
		Biodiversity strategy	Sustainable use of biodiversity
		Thematic strategy on the sustainable use of natural resources(p)	Sustainable use of natural resources

(a): Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC

(b): Directive 2004/35/CE of the European Parliament and of the Council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage

(c): Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora

(d): Council Directive 2009/147/EC of 30 November 2009 on the conservation of wild birds

(e): Council Regulation (EC) No 338/97 of 9 December 1996 on the protection of species of wild fauna and flora by regulating trade therein

(f): Commission Communication of 22 May 2006 “Halting the loss of biodiversity by 2010 – and beyond – Sustaining ecosystem services for human well-being” COM(2006) 216

(g): Communication from the Commission to the Council and the European Parliament of 4 February 1998 on a European Community biodiversity strategy COM(1998) 42

(h): Commission Communication of 27 March 2001 to the Council and the European Parliament: Biodiversity Action Plan for the Conservation of Natural Resources (Volume II) COM(2001) 162

(i): Commission Communication of 27 March 2001 to the Council and the European Parliament: Biodiversity Action Plan for Agriculture (Volume III) COM(2001) 162 (j): Council Decision 82/72/EEC of 3 December 1981 concerning the conclusion of the Convention on the conservation of European wildlife and natural habitats (Bern Convention)

(k): Council Decision 93/626/EEC of 25 October 1993 concerning the conclusion of the Convention on Biological Diversity

(l): Commission Communication of 22 September 2006 entitled “Thematic strategy for soil protection” COM(2006) 231

(m): Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy

(n): Regulation (EC) No 1107/2009 of the European Parliament and of Council of 21 October 2009 concerning the placing of plant protection products and repealing Council Directives 79/117/EEC and 91/414/EEC. (o): Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides

(p): Communication from the Commission of 21 December 2005 – Thematic Strategy on the sustainable use of natural resources COM(2005) 670

With regard to Non-target organisms and NTO biodiversity, species assemblages and food webs in the crop should be considered, specifically describing the functional groups active in the receiving environments and how the situation could/could not be different in GM plant receiving environments based on laboratory and/or field studies on species at different trophic levels using the methods described in section 4.5 and in the EFSA opinion on NTO testing (EFSA, 2010b). Comparisons should be made with the range of current pest management strategies so that comparative estimates of biodiversity impacts of GM plants can be performed under a range of systems and related to current practices.

With regard to crop diversity, the need to protect crop genetic resources has sparked a growing interest in the genetic diversity maintained in traditional farming systems worldwide (Van Heerwarden et al., 2010). It is likely that GM crops, which have only one or a few genes changed, will continue to need to exploit the wide genetic diversity available in gene banks and breeding programmes around the world in order to optimise crop production and performance in a range of environments. As an example in should be noted that the MON810 event has been commercialised in over 100 different varieties in Europe in order to optimise crop performance in different regions and for different markets.

Soil biodiversity also consists of several trophic levels and numerous varieties of flora and fauna. Understanding the role of crop plants within the soil ecosystem provides the baseline for assessing any differing effects of GM plants. The close interaction between crop cultivation and soil processes means that soil organisms will directly and indirectly interact with GM plants and their products (eg Bt-proteins) released from GM crops. In any risk assessment the importance of the biodiversity of the system and the level of functional redundancy within the soil system needs to be addressed. FAO (2008) indicate that soil biodiversity can be assessed, managed and conserved, showing examples of successful and unsuccessful practices which have been used in various regions of the world to manage soil biodiversity. Moreover the Conference of the Parties to the Convention on Biological Diversity (CBD) identified soil biodiversity as an area requiring particular attention. It is therefore important that soil is specifically included in environmental impact assessments.

3.2.1 Environmental Damage

Environmental damage is defined as a measurable adverse change in a natural resource (e.g. of a protected species, ecosystem services or on other environmental entities of concern) or measurable impairment of a natural resource service which may occur directly or indirectly.

The following three principles merit attention for the testing of GMOs and/or ecosystem services (starting in the problem formulation phase):

1. “Damage or harm” means a measurable adverse change. This definition has implications for ERA in respect to practicality since there is a need to quantify the effects on biota in receiving environments.

2. The significance of any damage needs to be evaluated on a case-by-case basis particularly in relation to species population sizes and the potential for recovery of the affected biota. This definition has implications for the assessment of the magnitude of observed changes.

3. Representativeness of the receiving environment, protection goals and ecosystem services needs to be determined.

A crucial step in ERA problem formulation is to identify the aspects of the environment that need to be protected from harm according to environmental protection goals set out by EU or Swiss legislation (see e.g. BATS 2000, and Table 1 for EU legislation). Because protection goals are general concepts, they should be translated into measurable assessment endpoints. Defining assessment endpoints is necessary to focus the risk assessment on assessable/measurable aspects of the environment – a natural resource (e.g. natural enemies) or natural resource service (e.g. biological control functions of pest populations performed by natural enemies, or pollination) that could adversely be affected by the GM plant and that require protection from harm.

Subsequently, within the problem formulation, the identified potential adverse effects need to be linked to assessment endpoints in order to derive testable hypotheses that allow quantitative evaluation of the harm posed to those assessment endpoints. The hypotheses are of importance as they will further guide the setting up of a methodological approach on how to evaluate the magnitude of harm. Through hypothesis, assessment endpoints are translated into quantitatively measurable endpoints, termed measurement endpoints (such as measurements of mortality, reproduction, abundance). A measurement endpoint can be regarded as an indicator of change in the assessment endpoint, and constitute measures of hazard and exposure.

Finally, for each measurement endpoint, the level of environmental protection to be preserved is expressed through the setting of ‘limits of concern’ which may take one of two forms. For studies in the environment(s) that are controlled, the limits of concern will usually be trigger values which, if exceeded, will either lead to conclusions on risks or the need for further assessment in receiving environment(s). For field studies, the limits of concern will reflect more directly the minimum effect that is considered to potentially lead to harm. If these limits are exceeded then detailed quantitative modelling of exposure may be required to scale up effects at the field level both temporally and spatially. Limits of concern can be defined by literature data, modelling, existing knowledge and policy goals.

3.2.2 Environmental Scope

It is important to consider whether GM plants are released intentionally (via cultivation) or unintentionally (from imported products).

The major route of environmental exposure will be the intended release of GMP via cultivation. On a case be case basis unintended but unavoidable releases into the environment also needs to be considered, e.g. the loss and spillage of seeds from imported food/feed products. In many cases food and feed processing or handling renders seeds non-viable (e.g. Reuter et al., 2010b).

a. Unintended release (e.g. derived from imports of food and feed containing or consisting of GM plants)
If a GMP is not to be grown or cultivated in a country then the environmental risk assessement relates primarily to any exposure routes of the GM plant and its products through its intended use and any accidental release of the GMP into the environment during transport and use.

b. Intended release of GM plants for cultivation
If a GMP is to be grown or cultivated then the environmental risk assessment should be based on an assessment of all possible exposure routes associated with the growing, harvesting and processing of the GMP, the event and its products, and on the impacts of the cultivation of the GMP (Bartsch and Schuphan, 2002).

3.3 Receiving Environment

Accordung to EFSA (2010a) the receiving environment(s) is the environment into which the GM plant(s) will be released and/or into which the transgene(s) may spread either intentionally or unintentionally. The receiving environment(s) is characterized by 3 components:

- The GM plant (e.g. plant variety species, genetic modification(s) and intended uses(s)).
- The Geographical Zones (e.g. the climate, altitude, soil, water, flora, fauna, habitats…).
- The Management Systems (e.g. land use, and production systems, other cultivated GM plants, cultivation practices, integrated and other pest management, non-production activities and nature conservation activities).

In the component “Management Systems”, land use, production systems should be considered as these systems can differ significantly between geographical regions (e.g. irrigated maize versus non-irrigated cultivation elsewhere). Moreover, within a region, cultivation of GM plants for different purposes may also have certain specific risk assessment implications (e.g. green maize for biogas or silage with earlier harvest compared to grain maize).

These three components result in biotic and abiotic interactions that should be considered when establishing representative scenarios considering receiving environment(s) for carrying out the ERA of a GM plant. A broad range of various environments in terms of their flora and fauna, climatic conditions, habitat composition and ecosystem functions and human interventions occurs in the EU. Accordingly, GM plants will potentially interact with those differing environments.

The ERA should be carried out on a case-by-case basis, meaning that the required information may vary depending on the type of the GM plants and trait (s) concerned, their intended use(s), the potential receiving environment(s). There may be a broad range of environmental characteristics (regional-specific) to be taken into account. To support a case-by-case assessment, it may be useful to classify regional data reflecting aspects of the receiving environment(s) relevant to the GM plant (e.g. botanical data on the occurrence of wild relatives of GM plants in different agricultural or (semi) natural habitats of Europe, effects of production systems on the interactions between the GM plant and the environment).

Relevant baseline(s) of the receiving environment(s), including production systems, indigenous biota and their interactions, should be determined to identify any differences associated with the characteristics of the GM plant and their potential to cause harm. Defined relevant baselines refer to current production systems for which generally published literature is available. These baseline(s) serve as a point of reference against which future changes can be compared. The baseline(s) will depend to a considerable extent on the receiving environment(s), including biotic and abiotic factors (for example, natural preserved habitats, agricultural farmland or contaminated land). Databases (e.g. the Knecht et al. (2010) on maize field arthropods) may be helpful for comparing the species representing particular ecological functions and/or taxonomic groups in each country and thus can support ERA.

Both the plant and the transgenic trait(s) determine where the GM plant will most likely be grown. Some GM plants (e.g. cotton, rice) can realistically be cultivated in some geographical zones only, while others, like maize and potato, may be cultivated more widely in Europe. Transgenic traits such as biotic (e.g. pest resistance) and abiotic stress (e.g. drought and salt) tolerance will also define where GM plants are likely to be grown. Therefore, all these elements should be taken into account when defining the receiving environment(s) (e.g. considering geographical zones) for the ERA of each GM plant.

The ERA should take into account potentially harmful interactions of the GM plant with any other relevant GM plants that may have been deliberately released or placed on the market in the same receiving environments in the past, including interactions between the specific cultivation characteristics (e.g.repeated releases of the same GM plant, such as the use of plant protection products) associated with the different GM plants. In addition, applicants should consider likely and/or predicted trends and changes to receiving environments, including uptake of new technologies, and how these might interact with the GM plants. More detailed guidance on receiving environments is provided by EFSA (2010a).

4. Risk Characterization

Risk is characterised by combining the magnitude of the consequences of a hazard and the likelihood that the consequences occur (EC, 2002a). It is described in EFSA (2010a) as the quantitative or semi-quantitative estimate including attendant uncertainties, of the probability of occurrence and severity of harmful effect(s) based on problem formulation, hazard and exposure characterisation. The overall uncertainty for each identified risk should be described where relevant, possibly including documentation relating to:

- Assumptions and extrapolations made at various levels in the ERA;
- Different scientific assessments;
- Specified uncertainties;
- Conclusions that can be derived from the data.

The risk characterisation should indicate whether the problem formulation (including hazard identification), hazard characterisation and exposure characterisation are complete.

The following sub-chapters extract current knowledge for certain GM plant x trait combinations gained during the past ten or more years of research. These findings represent a general overview and therefore may not be fully applicable to new GM plant events.

4.1 Gene Flow, Persistence and Invasiveness of GM Plants

Recombinant genes introduced into crops conferring resistance to biotic and abiotic stress could potentially lead to increased fitness of the GM crop plants and of potential GM hybrids with related wild species (Chapman and Burke, 2006, Heuberger et al., 2010, Hooftmann et al., 2011, Raybould 2011, Yang et al., 2011, see also the reviews of Gressel, 2005, Ellstrand et al., 2010, Kwit et al., 2011, Snow and Campbell, 2011).

Inter- and intra- specific hybridization between vascular plants has been documented worldwide for decades. Gene flow has been widely documented for a range of crop species, and their wild relatives (Raybould and Gray, 1994, Darmency et al., 1998, Ellstrand et al., 1999, Ellstrand, 2001, Eastham and Sweet, 2002, Hails and Morley, 2005, Allainguillaume et al., 2006, Simard et al., 2006, Ellstrand et al., 2010, Zuo et al., 2011). The rate of hybridization between different species is highly dependent on parameters such as cross compatability, synchrony of flowering and the distance between populations (Ellstrand, 2003a, b).

Introgression of new genes into wild and feral populations will be initially determined by hybridisation frequency but also will depened on the fitness of the hybrids and subsequent backcross generations. Some crop genes found in wild relatives do not appear to confer fitness advantages so that there may be base rates of gene introgression dependant on factors such as pollination pressure/frequency and hybridisation frequency. Genes which confer fitness advantages would be expected to increase this rate of introgression and may subsequently result in uptake of genes to the extent that the behaviour of plant populations is altered. It is important that risk assessments consider whether changes in the fitness, persistence and invasiveness of GM plants will result in behavioural changes in the GM plants and compatible relatives with consequences for receiving environments. There is substantial variation in the rates and patterns of crop allele introgression after a single hybridization event. Certain crop alleles can introgress easily while others remain rare, supporting the assumption that neutral or beneficial transgenes that are not linked to maladaptive traits can persist in the wild (Snow et al., 2010). Frequent allelic introgression into weedy populations resulting from outcrossing with nearby cultivars determines the private alleles of local feral populations, possibly leading to their genetic differentiation. Introgression from a crop may play an important role in the adaptive evolution of feral populations (Jiang et al., 2011). However, despite a high expected frequency only few few F1 hybrids that produce seeds, and no backcrossed plants are found in nature for many plants (e.g. *Brassica rapa* and *B. napus*, Luijten and De Jong, 2011).

According to EFSA (2010a) the risk assessment considering persistence and invasiveness should conclude on (i) the impact of the GM plant and/or hybridising relatives in the production system, particularly through increased weediness and more intense weed control; (ii) the impact of the GM plant and/or hybridising relatives in semi-natural and natural habitats, through change in invasiveness or reduction of biodiversity or ecological function; (iii) why any anticipated harm may be considered acceptable; and (iv) what risk management measures may be required to mitigate any harm.

4.1.1 Altered Flowering Characteristics

Genetic transformation may alter flowering characters such as male fertility (e.g. in barstar/barnase oilseed rape), self and cross pollination potential, seed set, time of flowering, synchrony of male and female flowers etc. it is important that the consequences of such changes are determined in an ERA. Altered flowering characteristics played so far only a minor role in risk assessment practice, but future gene flow mitigation measures, based on molecular techniques, aim to alter flowering characteristics (Koziolek, 2009, see also chapter 6).

In one published study, a delayed flowering of Bt crops was observed (Hoheisel and Fleischer, 2007). The authors studied the effects of concurrent introduction of three vegetable Bt cultivars (sweet corn, potato, winter squash) with respect to coccinellid, aphid and pollen diversity. For all of the three GM varieties, the authors found a delay in pollen production in comparison to the isogenic non-genetically modified controls. Small differences were detected for sweet corn; larger differences were revealed for potato and squash. The production of substantial pollen amounts was one to three weeks delayed in the genetically modified lines. For the Bt potato event the peak of pollen production was one week delayed. The number of flowers was reduced in the Bt varieties but higher amounts of pollen were produced; the reason for this unintended effect is not known, and somaclonal variation might be one possible explanation. Altered flowering characteristics was also observed for some GM wheat lines in the NFP59 (Zeller et al., 2010).

In addition it is important to understand the significance of flowering characteristics in determining likely introgression of GM traits to wild populations. Stanton et al. (1986) studied the insect-attractiveness of white or yellow coloured flowers of the self-incompatible species *Raphanus raphanistrum*. According to their results, white coloured petals attracted fewer insects than yellow coloured. However, the maternal function in terms of fruit and seed production was not affected when comparing the seed set of white or yellow coloured flowers. Thus, the attractiveness of flowers and the frequency of insect visits do not seem to be sufficient explanation for female reproductive success when the male function of a flower is retained.

Raphanus sativus (cultivated radish) and *R. raphanistrum* (wild radish) are frequently used as a model system for studying the potential fate of GM traits in hybrids (Klinger and Ellstrand, 1994, Snow et al., 2001). Lee and Snow (1998) carried out a study on hybridization between cultivated radish (as potential target for genetic modification) and wild radish in view of potential fitness effects on hybrids. Cultivated radishes were white-coloured; the wild partner R. raphanistrum had yellow flowers but is known as polymorphic for flower colour (white or yellow). The hybrids invariably had white or pink coloured flowers like the crop partner, thus visualizing the transfer of crop genes. From pollinator studies, the authors found that the white coloured hybrids were significantly less frequently visited by pollinators than the wild yellow coloured parental plants. Consequently, a lower seed production was observed in the hybrids. The discrimination tendencies for the white-coloured hybrids were of substantial importance when hybrids were of great numbers. The results give evidence that pollinator preferences may potentially affect the likelihood for reproduction and survival of GM hybrids. However, as found by Stanton et al. (1986), the frequency of pollinator visits alone is not a sufficient estimate for reproductive performance.

Beside petal colours as factor for attracting pollinators, also volatiles emitted from the flowers to ambient air play an important role for orientation of insects and plant pollination (see e.g. review by Dicke & Baldwin 2010). Plants synthesize and emit a large variety of volatile organic compounds; some are probably common to almost all plants whereas others are specific to only one or a few related taxa (Pichersky and Gershenzon, 2002). Plant flavours are not only produced to attract pollinating insects; several plant species emit volatile substances acting as repellents to deter pests or herbivores from attacking the specific plants (e.g. scents of distress = “Herbivore-induced Plant Volatiles” – HIPV). Additionally, HIPV are attracting the herbivores enemies (predators) in terms of providing biological defence (Turlings and Ton, 2006).

Vogler et al. (2009) found in four terpenes and an aromatic compound differences in emissions between a GM apple line and two non-GM apple cultivars, but the volatiles were in the range of variability recorded for crucial references.

Pierre and Pham-Delègue (2000) demonstrated in a study on honey bee that the attractiveness of GM oilseed rape, constitutively expressing a protease inhibitor or HT (glufosinate), will not alter the attractiveness of such plants compared to non-GM plants. The analyses of nectar and flower volatile compounds from protease inhibitor plants discovered differences in the chemical composition between GM and non-GM oilseed rape. However, this did not change the attractiveness of the flowers for bees.

Altering flowering characteristics can be used to manage or prevent gene flow and this has been extensively researched in relation to hybrid seed production and coexistence of GM and non-GM crops (Hüsken et al., 2010). Some methods are discussed in Section 6 on Coexistence.

Conclusions regarding altered flowering characteristics:

- Altered flowering characteristics are theoretically possible but do not seem to play an important role in risk assessment of currently available GM crops.
- In the future, intended alteration of flowering characteristics may play an important role in managing plant-to-plant gene flow (see Section 6 on Coexistence).

4.1.2 Increased Fitness of GM Plants

Fitness is defined as the ‘contribution made to a population of descendents by an individual relative to the contribution made by others in its present population’. It is the relative contribution that an individual makes to the gene pool of the next generation (Begon et al., 2005).

Fitness is thus a relative and not an absolute term. It depends specifically on the biotic and abiotic environment for a given plant species or plant population. Various numbers of stressors affect growth, yield and reproduction of GM and non GM crops. Important environmental stressors are: weeds competing for space and nutrients on fields, pests feeding on crop plants like dwelling insects, infectious bacteria, fungi or viruses (Chapman and Burke, 2006). Consequently, fitness improvement might be provided by any transgene that affords protection against the stressors mentioned. In addition, traits conferring advantages for crop cultivation under detrimental abiotic conditions (e.g. drought) would also have the potential for enhancing fitness (Hails and Morley, 2005, Snow et al., 2005).

In case of hybrids containing a GM trait conferring tolerance against a specific stressor, the cost of expressing the extra protein is balanced by the advantage of the GM trait. However, the advantage does not appear if the pest or abiotic stressor is absent (Chapman and Burke, 2006, Snow et al., 1999). In this case the GM plant expression of the proteins may have a “fitness costs” or “cost of resistance” (Coley et al., 1985). Bergelson (1994) found reduced fecundity of transformed lines in comparison to untransformed plants in cases where the stressor was absent. However, the resistance costs vary substantially and Strauss et al. (2002) found costs ranging from 6 to 45%. Additionally, it was found in the below mentioned investigations by Vacher et al. (2004) on hybrid fitness of Bt protein expressing *B. napus x B. rapa* hybrids, that vitality of the Bt protein containing hybrids was reduced in the absence of the herbivores.

Where the genetically modified crop does not have any wild relatives in Europe, the ERA is simplified: only the fitness effect of the GM traits on the crop itself requires an assessment. Matters of environmental importance are factors such as the occurrence of volunteer or feral populations.

Where the GM crop has compatible wild relatives in Europe, it is important to determine the spread and establishment of the transgene in wild relatives in areas close to GM crops and in more remote areas and to determine the consequences for these populations and any associated adverse environmental impacts.

Stress tolerance and fitness

GM crops are being developed with improve abiotic stress tolerance to factors such as salinity or drought (e.g. maize), or tolerance against herbicidal agents (e.g. maize, soya cotton, oilseed rape etc…).

Drought and salinity are important environmental constraints to crop productivity in arid and semi-arid regions of the world. Currently, there are several attempts to improve drought tolerance in major crops like maize, potato, and soybean (Huang et al., 2000, Ahmad et al., 2007, Wu et al., 2008, Xue et al., 2007). The potential ecological impact of increased salt and drought tolerance for sugar beet was discussed by Bartsch et al. (2003), but no experimental data on potential drought or salinity resistant GM sugar beet are yet available.

At the same time, basic research has studied model plants, e.g. GM plants exhibiting increased tolerance to salinity in tomato (Foolad 2004) or perennial ryegrass (Lolium perenne; Wu et al., 2005). In both cases the gene used is expressing a vacuolar anti-porter protein controlling Na+/H+ antiport derived from *Arabidopsis thaliana*. In potato drought resistance is achieved by introduction of the trehalose 6-phosphate synthase (TPS 1) gene from *Saccharomyces cerevisiae* (Yeo et al., 2000). By introducing additional trehalose level controlling genes, tobacco plants were genetically modified to achieve drought tolerance (Karim et al., 2007). Genetically modified plants overexpressing trehalose-6-phosphate synthase (TPS) for drought tolerance are often not sufficiently protected against drought stress, due to either short-term trehalose accumulation or to pleiotropic growth aberrations. A better drought tolerance was achieved by introducing a double construct consisting of TPS and trehalose-6-phosphate phosphatase (TPP) from *Sacchoromyces cerevisiae* and combining it with specific regulatory elements from *Arabidopsis thaliana*. In this case plant growth was normal at certain levels of stress due to a still unknown water retention effect that was not found in the non-transformed varieties.

Genes conferring tolerance against specific non-selective herbicides are currently among the most common traits introduced into GM plants.Today, there are several different HT systems available on the market: introduced GM tolerance (eg ALS, 2,4D glyphosate, and glufosinate-ammonium tolerance) and non-GM technologies (e.g. Imidazolinone – Clearfield technology and Atrazine).

The herbicide tolerance trait offers fitness advantages in the presence of the complementary non-selective herbicide.

It is likely that volunteer HT plants will occur in successive years on the cultivation fields. However, herbicide-tolerant volunteers are of lesser importance if GM crops are cultivated in rotations with non-herbicide tolerant crops or crops expressing different herbicide tolerance traits. HT varieties (including HT oilseed rape or HT sugar beet) are also likely to have growth advantages on disturbed ground outside the cropping field if the specific herbicide is applied there. According to the specific national herbicide regulations this may apply for field and road verges or for railway tracks.

Watrud et al. (2011) conducted small-scale experiments on the consequences of glyphosate drift into field edges and roadsides. Such a drift can contribute to persistence of GM plants that express the CP4 EPSPS transgene due to higher fitness in these managed conditions (see also chapter 4.7).

Herbicide resistance development has also been reported in weeds associated with HT crops. Repeated cultivation of GM crops with the same herbicide tolerance puts an increased selection pressure on weed populations in fields, resulting in an increased likelihood of herbicide resistance evolving (Chapman and Burke, 2006). Currently, herbicide resistance to glyphosate has been observed for weeds like *Lolium rigidum* by Powles et al. (1998) and for *Conyza canadensis* by Koger et al. (2004). Up to now, more than 9 non-GM wild plants (weeds) resistant to glyphosate have been confirmed on a worldwide scale (Heap, 2011, Powles, 2008).

A consequence of the development of herbicide resistance in weeds has been an initial increase in the use of the specific herbicide (e.g. higher doses or more applications) and the use of mixtures of herbicides with the specific herbicide. In some cases farmers have used alternative herbicides or have changed their cropping systems. These changes in herbicide use have resulted in different environmental impacts and in some cases greater adverse impacts. The environmental impacts of herbicide management of GM crops are discussed in section 4.7.

Biotic stress tolerance and fitness

Resistance to certain biotic stressors in GM plants is being developed particularly against insect pests, pathogenic fungi and viruses. Studies on the consequences for the fitness of GM plants can be grouped into these main areas:

i. Pest tolerance and plant fitness

Field experiments with Bt maize expressing resistance genes against infestations of European Corn Borer (ECB) in infestation areas usually revealed high protection of the crop (Koziel et al.,1993). Beneficial effects in terms of yield are highly dependent on the local situation. In Europe, seed yield increases due to greater seed production and specific weight in infestation areas of up to 10% have been noticed.

Stewart et al. (1997) found higher numbers of seeds produced by insect protected Bt oilseed rape than by non-GM oilseed rape under the pressure of herbivores. Fitness in terms of seed productivity of GM hybrids with *B rapa* was also enhanced by Bt traits.

Similar results were obtained by Vacher et al. (2004) when testing the fitness of F1 hybrids between Bt protein expressing oilseed rape and *B. rapa*. Under permanent herbivore pressure Vacher et al. (2004) reported a significantly higher number of offspring from F1 hybrids between Cry protein expressing oilseed rape and *B. rapa* than from conventional *B. rapa* populations. Interestingly, the Bt-hybrids produced 6.2-fold fewer seeds than non trangenic plants in the absence of herbivores resulting in a remarkable decline of Bt-hybrids in the following generation. If herbivores were present the GM-hybrids produced 1.4 times more seeds than non-Bt hybrids. The authors conclude that Bt expressing hybrids will primarily benefit from the recombinant gene in terms of invading into wild floras when herbivore pressure is of high importance. The results of a hybridization experiment carried out by Hauser et al. (1998a; b) with non-GM hybrids of *B. napus x B. rapa* demonstrate, that the fitness of F1 plants was intermediate in comparison to their parents and declined further in F2 and backcross hybrids.

Mikkelsen et al. (1996) detected high similarity of GM *B. napus x B. rapa* hybrids to wild *B. rapa* plants with respect to morphology and chromosome number. F1 hybrids of *B. napus* x *B. rapa* had a relatively high fertility in comparison to the wild ancestor. However, the male fitness of the F1 hybrids was low in another hybridization experiment with glufosinate-tolerant oilseed rape and *B. rapa* (Pertl et al., 2002). Similar results were published by Halfhill et al. (2005) when crossing Bt protein expressing *B. napus x B. rapa*. The authors found the F1 hybrids and the backcrosses to be of similar nitrogen efficiency as oilseed rape. The hybrids were weakened in terms of competitive ability in comparison to wild *B. rapa*. Hence the assumption can be drawn that a diminished competitive ability would decrease their chances to spread and thus to persist in nature.

Snow et al. (2003) crossed Cry1Ac expressing cultivated Bt sunflower (*Helianthus annuus*) with wild sunflower and reported that the (GM) hybrid sunflower was more resistant to attacks of certain lepidopteran species. Since wild sunflowers also suffer from herbivory by lepidopterans, the transgene transferred to the hybrids had a significant resistance effect, reducing stem damage and flower head damage, and increasing seed set.

ii. Pathogen resistance and fitness

Burke and Rieseberg (2003) examined GM sunflower expressing the oxalate oxidase (OxOx) gene protecting the recombinant plants against *Sclerotinia sclerotiorum*. After backcrossing the gene into wild sunflowers over three generations no fitness effects in the recombinant hybrids could be detected, despite severe pathogen pressure. Seed production was not altered. Even non transformed and infested control plants were able to produce seeds at the same level as the recombinant plants. However, the effects were site dependent, giving rise to the assumption that environmental parameters have influenced the results.

No altered survivability (including the offspring) have been reported from studies with GM potatoes with increased pathogen tolerance (Rasche et al., 2006a, b and references therein).

Experiments with genetically modified and near isogenic virus (Rhizomania) resistant hybrids of sugar beet x wild sea beet demonstrated that biomass production depended on the intensity of virus infections and on the intensity of competition by other weeds (Bartsch et al., 1996). Virus resistance offered a fitness advantage only under specific conditions i.e. a high degree of virus infection. Wild forms of cultivated beets and wild sea beets seem to be naturally virus resistant (see review by Bartsch et al., 2003).

Conclusions regarding increased fitness of GM plants:

- Data are currently scarce on the long-term consequences of enhanced fitness effects of GM crops or GM hybrids expressing salinity or drought resistance genes. Currently, information for ERA has to be derived from analogous data on the behaviour of conventional crop varieties selected for salinity or drought resistance and wild species that might occur in dry or saline areas.

- Biotic stress tolerance induced fitness enhancement is case-specific for GM plants and their hybridising relatives and the environments where populations are currently restrained by biotic stress and where selective pressures can favour the GM trait (Arnold and Hodges 1995, Chapman and Burke 2006, Ellstrand et al., 2010).

- Specifc studies are therefore needed on all new stress tolerant GM plants and their wild relatives in order to determine changes in their fitness and any environmental impacts.

- Herbicide tolerance genes will only enhance fitness in cropping systems where the complementary herbicide is applied.

4.1.3 Outbreeding Depression

From the population biology point of view, the term “outbreeding depression” describes hybrids being offspring from crosses between individuals of a source and a recipient population that have lower fitness than progeny from crosses between individuals from the same population. The drop in fitness may be caused by insufficient homology of the genomes resulting in detrimental effects on the functioning of metabolic pathways or other unfavourable parental gene combinations undermining the hybrid’s vigour (Hails and Morley 2005). The ultimate consequence of these effects may be local extinction of wild species populations (Ellstrand et al., 1999). Outbreeding depression is of increased interest particularly within conservation genetics of rare and endangered wild plant populations. In general, knowledge of the consequences of inbreeding and outbreeding depression in wild populations as well as the capacity of small populations to adapt to local environmental conditions is urgently needed (Pertoldi et al., 2007). Since crop-wild hybridization ability is the prerequisite condition, these effects are specifically reviewed for the two crops with potential for hybridization with wild relatives in the EU: oilseed rape and sugar beet (See Sections 4.2).

Large scale cultivation of GM crops can result in repeated or continuous outcrossing and gene flow into populations of wild plants (Rhymer and Simberloff 1996, Ellstrand et al., 1999). Small wild populations could have high levels of outcrossing due to gene swamping and this could result in a high uptake of the transgene and changes in the fitness of the population. The risk of extinction of small crop-related populations will be higher if the GM crop is introduced into new areas since the wild populations still exhibit a higher degree of genetic ‘integrity’ than wild populations growing in production zones which have aleady introgressed crop genes into their populations (Ellstrand 2001). The receiving environment is also an important factor in determining whether outcrossing will result in depression of populations to levels of local extinction (Campbell et al., 2006, 2007). There are some indications for outbreeding depression in US approved GM squash crops hybridizing with wild relatives. Data of Fuchs et al. (2004) suggest that *Cucurbita texana* acquiring transgenes leading to virus resistance upon hybridization and introgression lead to such outbreeding depression under virus free conditions. A selective advantage was only observed if viruses like CMV, ZYMV, and WMV were severely limiting the growth and reproductibility of wild squash populations.

Conclusions regarding outbreeding depression of the GM cultivar:

- Outbreeding depression is of increased interest particularly within conservation genetics of rare and endangered wild plant populations that are related to GM crops.
- There are currently only limited data available for currently approved GM crops concerning adverse effecs resulting from outbreeding depression in the EU.
- Outbreeding depression is potentially a problem for wild relatives of GM plants such as sugar beet, oilseed rape and others and should be studied for each new GM trait being developed for Europe.

4.2 Specific Considerations for Crops of Interest to Switzerland

4.2.1. Oilseed Rape (*Brassica napus*)

Oilseed rape has the potential to distribute pollen and seeds over large distances and has seed that can persist for many years in soil presenting a potential weed problem. GM oilseed rape is currently imported into the EU for processing but is not approved for cultivation. Devos et al. (2011) concluded that feral GM HT plants resulting from seed import spills will have little relevance as a potential source of pollen or seed for GM admixture.

OSR is a potential weed of other crops in arable rotations and GM oilseed rape can contaminate conventional OSR crops through cross pollination, seed admixture and volunteers in following crops. Oilseed rape is generally regarded as an opportunistic species, and not as an environmentally hazardous colonizing species (Warwick et al., 1999). Populations of oilseed rape as well as hybrids between oilseed rape and *Brassica rapa* can persist outside of cultivation especially in disturbed soils (Crawley and Brown 1998, 2004, Pivard et al., 2007, Knispel et al., 2008). Pascher et al. (2010, 2011) and Squire et al. (2011) reported that feral oilseed rape shows a widespread capacity to persist in many EU regions and retain traits from varieties no longer grown. However, feral oilseed rape is not a relevant source of impurity at its present density in the landscape but provides opportunity for genetic recombination, stacking of transgenes and the evolution of genotypes that under strong selection pressure could increase and re-occupy fields. The latter case could lead to a coexistence problem e.g. via economic weed burden and impurity in future crops (see chapter 6).

However, any fitness advantage derived from new traits could increase the persistence in natural and semi-natural environments and would enhance the rate of gene flow to wild relatives.

A number of hybridization studies are available for oilseed rape and its wild relatives. *Brassica napus* is able to cross with its two genetic ancestors *Brassica rapa* (Warwick et al., 2003) and *Brassica oleracea*. In addition oilseed rape can hybridise with *Raphanus* and other *Brassica* species such as *B. nigra and B. juncea* (Chèvre et al., 2004). Di et al. (2009) reported that there is no fitness cost in hybrids of Bt GM oilseed rape and *B. juncea*, which might allow the transgenes to persist in ecosystems. Thus, these species can be regarded as an important crop/weed/wild plant complex for the potential environmental spread of GM traits.

Brassica rapa populations grow sympatrically with oilseed rape at several locations in Western Europe. Additionally, populations of *B. rapa* (as weeds as well as wild populations) are more common than wild *Brassica oleracea* populations (Wilkinson et al., 2003). Hybrids from *Brassica napus* and *B. rapa* have been studied in more depth in Denmark and the UK (Jørgensen and Andersen, 1994, Ammitzbøll et al., 2006). Allainguillaume et al. (2006) studied the fitness of spontaneous F1 hybrids between oilseed rape and *Brassica rapa* as well as of F2 hybrids between F1 offspring and *Brassica rapa* (first backcross). The authors estimated approximately 7.000 second generation hybrids (i.e. ~ 20% of F1 hybrids) occurring every year in UK. These could be transgenic if the crop crossing partner is GM.

At present only herbicide tolerant (HT) and male sterility and fertility restorer traits are being commercially exploited in GM oilseed rape. Several field studies and model predictions have reported that the presence of herbicide tolerance in oilseed rape does not change this behaviour or confer a fitness advantage, unless the respective herbicide is applied (Crawley et al., 1993, 2001; Fredshavn et al., 1995; Warwick et al., 1999, 2004; Norris and Sweet, 2002; Claessen et al., 2005a,b; Simard et al., 2005; Garnier and Lecomte, 2006; Garnier et al., 2006, 2008, Simpson et al., 2006). Moreover, there is no evidence that tolerance to glyphosate or glufosinate enhances seed dormancy, and hence the persistence of feral oilseed rape populations (Claessen et al., 2005a,b; Lutman et al., 2005a,b, Hall et al., 2005, Aono et al., 2006). Because glyphosate/glufosinate-containing herbicides are not widely used in ruderal ecosystems in Europe, feral oilseed rape plants ensuing from GMHT oilseed rape would not show any enhanced fitness and would thus behave as conventional plants. Only where and when the associated herbicides are applied, is a fitness advantage likely (see Watrud, 2011).

However GMHT feral oilseed rape and hybridised/introgressed relatives will develop in agricultural systems becoming weeds of oilseed rape and other crops. Changes in management will be needed to control these weeds and volunteers, particularly in non-GM systems wishing to remain below thresholds for adventitious presence. However they can be managed by the use of other herbicides and/or adequate mechanical and rotational practices (Beckie et al., 2004; Devos et al., 2004; Warwick et al., 2004; Légère, 2005; Simard et al., 2005); see also section 4.7 on impacts of cultivation and section 6 on coexistence.

In Canadian regions where GM oilseed rape is frequently grown, feral oilseed rape and *Brassica rapa* populations were shown to actively outcross with cultivated populations of GM oilseed rape and to accumulate transgenic traits (Beckie et al., 2006, Knispel et al., 2008). Other studies have shown the potential for GM oilseed rape to outcross and eventually disperse genes to cross-compatible plants such as *Brassica rapa* and *Raphanus raphanistrum* (Scheffler and Dale, 1994; Eastham and Sweet, 2002; Chèvre et al., 2004; Warwick et al., 2004, 2008; Claessen et al., 2005b; Jørgensen, 2007; Warwick et al., 2008; Devos et al., 2009a,b,c) in both Europe and North America. Hybridization between oilseed rape and wild radish (*Raphanus raphanistrum*) or cabbage (*Brassica oleracea*) may lead to viable offspring, even though to a relatively low extent (Darmency et al., 1998, Al Mouemar and Darmency 2004, Chèvre et al., 2004, Halfhill et al., 2004, Ford et al., 2006). F1 hybrids derived from *B. napus x R. raphanistrum* exhibit low fertility (Chèvre et al., 1997). Guéritaine et al. (2002) backcrossed GM oilseed rape to *R. raphanistrum* six times. Depending on the direction of the backcrosses, the fitness of lines differed. Hybrids derived from backcrossing with the wild relative kept wild characteristics to higher extents. However, in the presence of the transgene, fecundity of the hybrids was generally reduced by ~ 50%.

Ammitzbøll and Jørgensen (2006) tested spontaneous hybridization rates between male-sterile GM *Brassica napus spp. oleifera* with *Raphanus raphanistrum* as wild relative species. The populations of wild radish came from France, Switzerland and Denmark. The offspring of the crosses with the French wild radish population was 100 % confirmed as being hybrids. From the cross with the Swiss population only 53 % were hybrids and only 2 % of the offspring crossed with the Danish population were confirmed as hybrids. Thus, the outcrossing barrier between different but related species seems to vary according to populations. Lee and Snow (1998) revealed the importance of pollinator preferences for the fate of *Raphanus* hybrids since the hybrids often show white coloured flowers, which are less attractive to pollinators.

None of these studies have shown changes in fitness in the introgressed plants and thus confirming the assumption that, in the absence of the associated herbicides, hybrids or wild relatives containing the herbicide tolerance trait would not show any enhanced fitness and would behave as conventional plants. However genetically modified *Brassica rapa* hybrids and backcross generations could become invasive if they contain fitness increasing traits. Allainguillaume et al. (2006) estimated that there is a decline in the fitness of hybrids, however F2 offspring was usually of higher fitness than F1 as the proportion of wild type genes increased.

Experiments with Bt oilseed rape and *B. rapa* have shown some fitness enhancement in the presence of high levels of target pest infestation (Stewart et al., 1997, Halfhill et al., 2005). However Bt oilseed rape has not been commercialised in any country to date.

Conclusions regarding gene flow, fitness and invasiveness of oilseed rape:

- Gene flow within some species of the genus *Brassica* and closely related genus can occur and has resulted in gene stacking in both feral rapeseed and in *B rapa* plants.

- Changes in fitness in Bt and introgressed plants has been shown when there is infestation with the specific insect pest.
- In the absence of the associated herbicides, GM plants, hybrids or wild relatives containing the herbicide tolerance trait do not show enhanced fitness and behave as conventional plants.

4.2.2 Sugar Beet (*Beta vulgaris ssp. vulgaris*)

The ancestors of cultivated sugar beet (*B. vulgaris ssp. vulgaris*) are the sea beets (*B. vulgaris* ssp. *maritima*) naturally growing along drift lines of sea shores. Since wild sea beet and cultivated varieties have the same ancestor and sea beets are still used as plant genetic resources in breeding programmes, both forms are often difficult to distinguish (Ford-Lloyd and Williams, 1975, Cureton et al., 2006). Cultivated *B. vulgaris* varieties, in contrast to wild varieties, are biennials, growing in the year of cultivation merely vegetatively as a result of breeding and selection. If the crop is not harvested in the first season, flowering usually occurs after the winter season, though sugar beets are frost-sensitive and may not survive cold winters. Interestingly, the life span of wild sea beet plants exhibits a range very different periods. Wild sea beet plants may survive up to 11 years in populations in Northern Brittany; whereas in populations occurring in Italy and south-western France the life time span of individual plants usually ends after the second season (van Dijk et al., 1997). Sugar beet hybridizes with their wild relatives in the seed production areas, leading to crop-wild hybrid weed beets. In sugar beet, the reproductive potential allows weed beet to restore and increase the soil seed bank of glomerules rapidly (Landova et al., 2010). The increased fitness of weed beets in sugar beet fields originates from positive selection on first-year flowering induction depending on genetic elements (e.g. the bolting B gene) inherited from the wild (Arnaud et al., 2011). However, the bolting tendency in the crop species *Beta vulgaris*, which includes sugar beet, is a complex trait governed by various environmental cues, including prolonged periods of cold temperatures over winter (vernalization) and photoperiod, and multiple genetic factors (Abou-Elwafa et al., 2012, Pin et al., 2012).

Premature flowering of cultivated varieties may occur after sowing in spring due to low temperatures acting as a late vernalization effect (Lavigne et al., 2002, Sukopp et al., 2005). In addition, sugar beets are capable of outcrossing other cultivated forms of *B. vulgaris* like red beet, leaf beet or table beet. Feralized annual sugar beets (weed beets) occur in fields and as feral populations growing in disturbed habitats in areas with maritime climates, and gene flow from GM beet has experimentally been proven up to 300 m (Saeglitz et al., 2000, Darmency et al., 2007, Darmency and Richard-Molard 2008) and has been demonstrated to occur over long distances (800 m) in conventional beet (Jensen and Bogh 1941). Interacting populations in the Genus *Beta* are within the species *B. vulgaris*, *B. macrocarpa*, *B. atripicifolia* and potentially also *B. trigyna*. Genetic material from sugar beet to wild or weed beet populations can be introduced via pollen, seeds or feralization of the cultivar. Human activities like seed production and seed trade contribute substantially to the amount of naturally occurring gene flow over long distances. Best management options to decrease unintended gene flow are bolter control in root production fields, and isolation distances in seed production areas (Bartsch, 2010).

In summary, gene flow may occur (i) between flowering cultivated beets and wild beet in coastal beet breeding areas, (ii) after vernalization and flowering of vegetative parts remaining on fields after harvest, (iii) by late vernalization and flowering of seedlings after sowing in spring, (iv) from adventitious bolters occurring during cultivation (Bartsch et al., 2003, Andersen et al., 2005, Sukopp et al., 2005). GM traits could potentially be transferred from cultivation areas far away from natural sea beet habitats by weed beets acting as bridging plants (Fénart et al., 2007).

Outbreeding depression was indirectly studied in experiments with GM (BNYVV resistant) hybrids of sugar beet x wild sea beet and near isogenic control hybrids (Bartsch et al., 1996). Decreased biomass production and competitiveness were observed for one of the two GM events studied in the absence of a fitness advantage (see review by Bartsch et al., 2003). The most likely explanation is an unintended effect due to the genetic transformation and that the observed effect was likely to be independent of the specific GM trait. Sugar beet breeders use wild populations of *Beta vulgaris* as sources of particular traits or genes quite commonly in their breeding programmes and thus their genotypes overlap quite considerably (Bartsch et al., 2003).

These results underline the importance of selection pressure for the expression of fitness effects of GM traits in oilseed rape and sugar beet. Without selection pressure, hybrids of interspecific crosses often exhibit reduced fitness (Arnold and Hodges, 1995, Allainguillaume et al., 2006). Outbreeding depression is thus a more general concern for crop-wild populations, for GM crop ERA the characteristics conferred by the new traits could be of importance.

A close relationship to maritime climates in Europe is obvious for both oilseed rape and sugar beet crop/wild plant complexes.

Herbicide tolerant (HT) sugar beet is cultivated in N America and has been tested in several European countries. The US authorities (USDA, 2011) filed in 2011 a comprehensive environmental impact assessment on this sugar beet. No increase in fitness of the GM beet has been reported though there are reports of increased performance and yield of GMHT beet due to the lack of phytotoxicity of the glyphosate herbicide to the HT beet compared with the sensitivity to conventional herbicides (Sweet et al., 2004).

The economics of sugar beet production, weed beet problems and the absence of detectable transgenic DNA in sugar make it likely that this crop could be cultivated in Europe in a few years. Carefull management of beet seed production will be required to ensure that crops contain no annual GM HT beets and crop management will be needed to prevent spread of transgenes into weed beets.

Conclusions regarding gene flow, fitness and invasiveness of sugar beet:

- Gene flow within the genus *Beta* can occur.
- None of these studies have shown changes in fitness and invasiveness of herbicide tolerance genes in the introgressed plants. Virus resistance genes might offer fitness advantage under certain levels of infection.
- In the absence of the associated herbicides, hybrids or wild relatives containing the herbicide tolerance trait do not show any enhanced fitness and behave as conventional plants.

4.2.3. Potato *(Solanum tuberosum ssp. tuberosum)*

Potatoes have been cultivated in Europe for about 300 years and today they are a widespread crop used throughout Europe as well as many other countries. Potato is being genetically modified for resistance to a range of pests and diseases and for changes in quality (e.g. modified starch). Virus resistant potatoes have been cultivated in N America. Bt potatoes with resistance to Colorado potato beetle and potatoes with resistance to nematodes and blight (Phytophthora spp) have been tested in many countries.

The organs of interest are the tubers produced in the soil. Plants are capable of vegetative reproduction via seed tubers and sexual reproduction occurs through flowering and fertile fruit set. Self-pollination rates are high (80-100% and many cultivars show low levels of fertility (OECD, 1997). Cross pollination is mostly limited to 3 m, maximum distances are 10 m (McPartlan and Dale, 1994). No gene flow has been found with the potato's wild relatives *Solanum nigrum* and *S. dulcamara* in the field (Eijlander and Stiekema, 1994, McPartlan and Dale, 1994, Conner, 1997). However, very low frequency of hybridization is possible with *S. nigrum* under artificial conditions (OECD, 1997). No data are available on potential transfer to other *Solanum* species, e.g. to *S. eleagnifolium*. Therefore hybridisation between cultivated *S. tuberosum* and other *Solanum* species appears very unlikely in Europe based on empirical knowledge and literature reports.

Potato tubers and seeds are frost sensitive but can survive if insulated by soil resulting in "ground keepers" in subsequent seasons. They are destroyed by a frost period of 25 h at -2°C or by a frost period of 5 h at -10°C (OECD, 1997). In applications for deliberate releases of GM starch potatoes the applicants frequently report on potential altered winter survival of the new potato lines. There is no report on alterations in frost-hardiness or overwintering for amylose- or amylopectine enriched lines.

Conclusions regarding gene flow, fitness and invasiveness of potato:

- Gene flow between cultivated *S. tuberosum* and other Solanum species appears very unlikely in Europe.
- Starch modification does not seem to offer fitness advantages and changes in invasiveness.
- No reports on fitness advantages of other GM traits are available so far.

4.2.4. Maize (*Zea mays*)

Maize has also been introduced to Europe about 300 years ago. It is a highly domesticated annual plant and cannot survive temperatures below 0°C for more than 6 hours after having reached the 5-leaf stage; before reaching this stage the susceptible growing point is still below soil surface (OECD 2003). Survival of maize inside and outside cultivation in Europe is mainly limited by a combination of poor competitive ability, absence of a dormancy phase, susceptibility to diseases and to cold climate conditions. Maize is an open-pollinated cross-fertilizing species – the self-pollination rate is about 1-5% – and thus the movement of genetically modified traits between maize plants is not easily controlled. Maize pollen grains being about 90-100 µm in diameter are among of the heaviest and largest wind-dispersed pollen grains (Ma et al., 2004). Maize pollen has the potential for movement over great distances like any other biotic or abiotic particle of similar size and weight, mostly depending on wind and temperature conditions (Eastham and Sweet 2002, Henry et al., 2003, Devos et al., 2005). The longevity of maize pollen viability ranges from 24 hours to several days depending on air temperature and humidity. Pollen loses viability in hot dry weather after 1 to 2 hours due to desiccation (Emberlin et al., 1999, Luna et al., 2001, Owen, 2005).

According to Emberlin et al. (1999) percentages of airborne pollen concentrations downwind compared with concentrations at 1 m from the source are approximately 2 % at 60 m, 1.1 % at 200 m and between 0.75 and 0.5 % at 500 m. However, such numbers are just rough estimates since dispersal gradients would be altered by climatic conditions and local topography. No wild relatives occur in Europe since maize is the only representative of the genus Zea. Vertical gene transfer is limited to other cultivated maize plants (OECD, 2003, Baltazar et al., 2005, Pineyro-Nelson et al., 2008, Bitocchi et al., 2009).

In addition, gene flow may also occur via seeds (Abbott et al., 2003, Snow 2002a,b). At harvesting and during transport individual kernels of maize are distributed. These can result in volunteers appearing in fields and along roadsides. However maize does not establish populations outside of cultivation (Gould, 1968). Volunteers of GM maize could lead to management problems if the GM maize is grown in rotation with non GM maize. Also GMHT maize may be adventitiously present in other HT crops cultivated in rotations which are tolerant to the same herbicide.

Genetically modified maize containing insect resistance (Bt) and/or herbicide tolerance (mostly to glyphosate) is widely grown around the world. Bt maize MON810 is now extensively cultivated in Spain and smaller areas of cultivation are expanding in other European countries eg Portugal, Slovakia, Romania, Serbia. Previously MON810 was cultivated in France and Germany but has now been banned on the basis of claims of environmental risks which have not been substantiated by the Scientific Committees providing risk assessment advice to the government in those countries or by EFSA. By contrast no adverse environmental effects have been reported associated with this cultivation and some benefits such as reduced insecticide usage (JRC, 2008) have been reported. However concerns about effects on non-target Lepidoptera remain. Recent assessments by the EFSA GMO panel considered that it was extremely unlikely that field and/or field margin populations of Lepidoptera would be exposed to toxic levels of Bt maize pollen from maize MON810 that would effect their populations (EFSA, 2009a) but that some extremely sensitive lepidoptera may be adversely affected by another Bt maize, 1507, in areas where this maize might be extensively cultivated (EFSA, 2011c, see also section 4.5).

Applications for maize NK603 containing glyphosate tolerance and a maize cultivar containing Bt and glyphosate tolerance have been assessed recently by EFSA and some competent authorities. Concerns were raised that the use of the herbicides could reduce farmland biodiversity and encourage development of weeds with resistance to these herbicides, as has been reported in USA. EFSA recommended that the herbicides should only be used in management programmes that maintain biodiversity and prevent weed resistance developing (e.g. EFSA, 2009b) – see section 4.6.

Maize with drought tolerance is currently being commercialised in USA. This maize will have fitness advantages in certain dryer environments but the consequences of this for European environments have not yet been assessed.

Conclusions regarding gene flow, fitness and invasiveness of maize:

- Vertical gene transfer is limited to other cultivated maize plants.
- Currently used GM traits (herbicide tolerance and insect resistence) do no offer fitness advantages and changes in invasiveness.
- Maize with drought tolerance will have fitness advantages in certain dryer environments but the consequences of this for European environments are unknown.

4.2.5. Soybean (*Glycine max*)

The cultivated soybean species (*Glycine max*) belongs to the subgenus Soja of the genus Glycine. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). In Europe, soybean is mainly cultivated in Italy, France and Romania and less in Hungary (FAOSTAT, 2005). Weedy soybean has not been reported growing naturally outside its centre of origin. This holds true for areas such as the Americas and Europe, where only cultivated soybeans are found (Lu, 2005).

Seed and pollen are potential sources of gene dispersal. However, soybean is an annual almost completely self-pollinating crop in the field with a percentage of cross-pollination usually lower than 1% (Weber and Hanson, 1961; Caviness, 1966; Lu, 2005). Temporal and spatial isolation will be effective strategies to minimize hybridization between GM and wild soybean (Mizuguti et al., 2010).

GM herbicide tolerant soja is cultivated extensively in N and S America and some other countries (e.g. Ukraine, Serbia). It was also cultivated in Romania but was stopped when Romania entered the EU, as EU approval had not been given. An application for cultivation in Europe is currently being considered by EFSA (EFSA, 2012a).

Conclusions regarding regarding gene flow, fitness and invasiveness of soybean:

- Vertical gene transfer occurs at very low levels to other cultivated soybean plants only in Europe.
- Currently used GM traits (herbicide tolerance) do no offer fitness advantages and changes in invasiveness, except in the presence of the herbicide.

4.2.6 Wheat (*Triticum aestivum*) and Other Cereals

Wheat is largely self pollinating species with limited outcrossing. Some outcrossing to wild related grasses such as *Aegilops speltoides, Ae. Biuncialis, Ae. Geniculata, and Ae. triuncialis* has been reported. The risk assessment of GM wheat cultivars needs to evaluate the importance of amphiploids as a bridge for transgene introgression and for gene escape to the wild (Loureiro et al., 2009).

GM wheat with herbicide tolerance, disease resistance and modified grain quality characters has been developed in several countries. There has been reluctance to commercialise GM wheat because it is a primary food crop and there are concerns about consumer acceptance. However recently some of the producer and processing organisations in several wheat growing countries (eg USA, Argentina, Canada, Australia) appear to have reached agreement in principle that they will accept GM wheat.
If glyphosate tolerant GMHT wheat was developed it would be cultivated in rotation with glyphosate tolerant rapeseed and other crops which would create management problems. In addition while glyphosate would give effective control of grass weeds, there are concerns that glyphosate tolerance would move into Goat Grass (*Aegilops* spp) making it more of a problem weed to control (Arrigo et al., 2011, Econopouly et al., 2011).

Disease resistant GM wheat was the focus of a major study of agronomic and environmental impacts in the Swiss NFP 59 programme (Foetzki et al., 2011).

There is little GM work at present on other cereal crops such as barley, oats, triticale and rye. It is likely that developments made in wheat will be replicated in these crops but not in the forseable future. There are extensive research and field trial programmes in Australia and some other countries for the development of drought tolerant wheat.

Conclusions regarding regarding gene flow, fitness and invasiveness of wheat and other cerals:

- Vertical gene transfer is limited to other cultivated wheat plants and some wild *Aegilops* species.

- Currently used GM traits (herbicide tolerance) do no offer fitness advantages and changes in invasiveness, except in the presence of the herbicide. The ecological impact of other GM traits (eg drought tolerance) has not been studied so far.

4.2.7 Trees and Fruits

A number of GM trees have been developed and field tested in the last 15 years. These carry novel or modified traits such as herbicide and insect tolerance, low lignin content and long fibres for pulp and paper production, heavy metal accumulation for phytoremediation, virus resistance, and fast-growing varieties for biomass production (Boerjan et al., 2003, Busov et al., 2005a,b, Ražanskienė et al., 2005, 2006, Robischon, 2006, Fladung, 2011). Because of the long life cycles of trees and their particular significance in many terrestrial ecosystems, concerns of potential impact of transgenic trees have been raised (Slavov et al., 2004, Wennström, 2004, OECD, 2006, Fladung et al., 2012). The ERA of trees has to consider their long life spans and cycles, their ability to disperse genes through seed and pollen over large distances. They form both the pioneering and the climax vegetation in a diverse range of natural habitats and are a major component of these environments providing niches for a range of species as well as food. Thus determining environmental impacts is made difficult by these complex interactions operating over large time scales. These impacts will include changes to the behaviour of GM trees as well as direct and indirect consequences of spread of transgenes via vertical or horizontal transfer pathways and the pleiotropic and unintended effects of the altered expression patterns of the transgenes.

Research on biosafety of GMTrees has already been initiated in a number of European countries (e.g. Belgium, Netherlands, Germany, Finland, France, Spain, Sweden, UK and others). This research included the potential impacts of outcrossing, the development of containment strategies, and the relationship between the genomic position of the transgene and its stable expression. Other studies have been considered principally the alteration of the transgenic host plant genome for the possibility that these changes can also cause pleiotropic, often undesirable alterations in plant metabolism and physiology. Also effects on composition and activities of soil microbial community as well as the fate of the transgene and proteins from GMTs in soil were monitored (Pilate et al., 2002, Kaldorf et al., 2002, Pasonen et al., 2005, Fladung and Ewald, 2006, Hönicka and Fladung, 2006a,b). Genetic modification can alter senescence and winter dormancy in GM poplar according to Hörnicka et al. (2008). In Canada and China, field trials with transgenic poplar (*Populus* spp.) have been conducted to study the stable integration and expression of the inserted genes (Donnarumma et al., 2007a,b, 2008). China established commercial plantations of insecticide-producing varieties a few years ago and it is likely that transgenic trees will become planted as commercial crops in plantation forestry and increased number of trials and releases for commercial plantations can be expected (Robischon, 2006, Fladung, 2011, Harfouche et al. 2011, 2012, Viswanath, V. and Strauss, S.H., 2011).

There are two main technological challenges for GMT tree development:

i) the establishment of efficient containment strategies, and

ii) the development of methods leading to predictable/reproducible integration of foreign genes in the plant genome.

i) Containment strategies have already been proposed including the establishment of male and/or female sterility as well as inhibition of vegetative propagation (Sederoff, 2007, Strauss et al., 2004, Hönicka and Fladung 2006). ii) The integration site of the transgene in the genome affects the stability of the expression of this transgene, partly due to methylation and silencing events. In addition, depending on the molecular layout of the integration locus as well the possibility that alternative splicing of the foreign gene irrespective of its origin occurs, the production of “variants” of the intended protein is theoretically thinkable. In this context, the targeted integration to known genomic position appears to be a valuable approach. Although gene targeting has been shown to be possible in plants, albeit with a lower frequency, so far nobody has developed this commercially.

A range of small, cane and tree fruits have been transformed with a range of characters including pest and disease resistance, increased production and modified quality characteristics. GM virus resistant papaya was first developed in Hawai and is now being cultivated in several countries. Similarly virus resistant GM melon is also being cultivated in USA and elsewhere. Considerable research and development work is being conducted on a range of other fruits including strawberries, raspberries, blueberries, grapevine, apple and plum. In the case of the last three there is interest in developing GM rootstocks which confer pest and disease resistance to the scion but allow the fruit to be sold as non-GM. A swiss team are involved in developing disease resistance in apples and other teams studied the environmental impacts of GM strawberry as part of the NFP59 programme.

Many of these fruits occur as wild types (e.g. *Fragaria*, *Ribes*, *Rubus*, *Prunus*) and most have both wild and cultivated relatives with which they can hybridise. Thus changes in plant characteristics and gene flow and its consequences are the primary concerns in environmental risk assessment of GM fruits and trees. Because they are perennial species with perennial relatives, risk assessments must consider long term consequences of GM induced changes in plant characteristics. In addition integrated pest management is widely practised in fruit production so that it is important to understand how GM crops will interact with beneficial biota, particularly predators and pollinators.

Conclusions regarding regarding gene flow, fitness and invasiveness of trees:

- Vertical gene transfer is in most case widespread within the same Genus.
- Environmental risk assessments must consider long term consequences of GM induced changes in plant characteristics
- More conclusions are not possible as experience is lacking.

4.2.8 Vegetable Crops

A range of GM plants have been developed from a wide range of vegetable species (Srivastava et al., 2011). The introduced traits include pest and disease resistance and changes in productivity, development and quality. Virus resistance has been developed in a range of cucurbitae and solanaceae and some commercialisation has occurred of virus resistant squash and peppers in USA and China. A range of fungal and bacterial resistance genes are being studied in several species. Bt insect resistance genes have been introduced into a range of species including Brassicae and aubergines but there is no commercialisation of Bt vegetables to date.

Genes to control plant development and quality have been tested in several species. The classic example is the slow ripening tomato which allowed later harvesting of fruits with higher sugar content and longer shelf life and enhanced flavour. These Flavr Savr tomatoes were commercialised in USA and widely sold in many countries including Europe, but are no longer marketed.

Most vegetable crop types are highly bred and adapted to cultivated conditions and thus have a low tendancy for ferality. However it will be important in an ERA to determine whether plant characteristics are changed to the extent that GM plants and their wild relatives will be more persistant or invasive outside of cultivation.

Many vegetable production systems are based on integrated crop, pest and disease management sytems so that it is important to understand how GM crops will interact with beneficial biota, particularly predators and pollinators. This is particularly the case for protected crops where biocontrol agents are being used.

Some vegetables have a high potential for outcrossing, particularly during seed production, and possess a range of compatible wild relatives. Thus gene flow to wild relatives and its consequences is an important consideration. Extensive studies have been conducted on species such as carrot (Hauser et al., 2004, Magnussen and Hauser, 2007) and lettuce (van de Wiel et al, 2004) to determine existing rates of gene flow from cultivated to wild types and between different wild types and populations. In these cases it can be anticipated that beneficial GM traits could be adopted by wild populations and so the consequences of such introgression will need to be determined.

Conclusions regarding regarding gene flow, fitness and invasiveness of vegetable crops:

- Vertical gene transfer is in many cases widespread within the same Genus.
- Enrivonmental risk assessments shall consider consequences of outcrossing to wild relatives
- More conclusions are not possible as experience is lacking.

4.2.9 Grasses and Forage Crops

Some grass species and alfalfa (lucern) have been transformed and consideration is being given to other species such as clovers and other grasses. GM herbicide tolerant alfalfa and creeping bent grass (*Agrostis stolonifera*) is now commercially available in the USA. The former is for forage production for livestock and the grass is to be used for amenity purposes on golf courses etc.. Other GM traits are being studied including pest and disease resistance and modified metabolism to produce forage with higher digestibility and conversion efficiency in ruminants, combined with lower methane emissions from the animals.

Most agricultural grasses, clovers and Medicago species are perennials and many occur as wild types as well as cultivated varieties. Grass has the potential to outcross both with wild relatives and also with some other species (each *Lolium* x *Festuca*). Similarly clovers and medics are outcrossing species. Gene flow from GM bent grass to wild relatives has already been reported in USA (Mallory-Smith and Zappiola, 2008). Fears about gene flow from GM alfalfa has resulted in several legal actions in USA and raised concerns about gene flow into Canada where GM alfalfa is not approved (Bagavathiannan et al., 2011a,b).

ERA of these species should therefore focus on changes in characteristics of the plants, changes in their associations with other biota and the consequences of gene flow to wild relatives and other species. In addition, where HT traits are introduced, the impacts of the use of the herbicides on biodiversity in pastures should also be examined.

Conclusions regarding regarding gene flow, fitness and invasiveness of grasses and forage crops:

- Vertical gene transfer is in many cases widespread within the same Genus.
- Enrivonmental risk assessments shall consider consequences of outcrossing to wild relatives.
- More conclusions are not possible as experience is lacking.

4.3 Plant-to-Microorganism (Horizontal) Gene Transfer

A non-sexual exchange of genetic material between organisms belonging to the same or different species is referred to as horizontal gene transfer (HGT). A prerequisite for such a gene transfer is the availability of pathways for the transfer of genetic material. HGT is a naturally occurring process occuring between bacteria (Wellington and van Elsas, 1992; Nielsen et al., 1998). The impact of the process depends on the likelihood of its occurrence and the magnitude of associated adverse outcomes (Nielsen et al., 1998; Droge et al., 1998). HGT, of genes related to antibiotic-resistant pathogens are profoundly important to human health. According to Allen et al. (2010) environmental reservoirs of resistance determinants are currently poorly understood. The origins of antibiotic resistance in the environment seem to be relevant to human health because of the increasing importance of zoonotic diseases as well as the need for predicting emerging resistant pathogens. Despite knowledge gaps, it is known that some organisms and some environments harbour antibiotic resistance genes irrespective of the human use of antibiotics (Allen et al., 2010).

Genomic DNA is a component of many food and feed products derived from GM crops including maize and soybean. It is well documented that DNA present in food and feed becomes substantially degraded during digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to microorganisms present in the digestive tract of humans, domesticated animals and other animals feeding on GM crops (e.g. EFSA, 2012b). Current scientific knowledge of recombination processes in bacteria indicates that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as plants to microorganisms) is not expected to occur at detectable frequencies under natural conditions (see EFSA, 2009c for further details). A successful horizontal transfer would require stable insertion of the transgene sequences into a bacterial genome and a selective advantage conferred to the transformed host. The only known mechanism that facilitates horizontal transfer of non-mobile, chromosomal DNA fragments into bacterial genomes is homologous recombination. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules and, in addition to substitutive gene replacement, facilitates the insertion of non-homologous DNA sequences if their flanking regions share sequence similarity with bacterial sequences in the recipient. A survey of Brigulla and Wackernagel (2010) on potential HGT from various GM plants indicates very rare transfer of foreign DNA. At the same time, in prokaryotic habitats, genes introduced into transgenic plants are abundant, and natural HGT frequencies are relatively high providing a greater chance for direct transfer instead of via transgenic plants. Brigulla and Wackernagel (2010) concluded that potential HGT from GM plants to prokaryotes is not expected to influence prokaryotic evolution and to have negative effects on human or animal health and the environment.

In soil, large DNA strands may be stabilized through the adsorption to soil constituents like clay, minerals or humic substances. Adsorbed DNA may persist for weeks or months (Gebhard and Smalla, 1999, Nielsen et al., 1997). Additionally, DNA may be preserved in dead plant tissue and kept biologically available to soil bacteria for hours, days or longer time spans.

The most likely scenario for the transfer of DNA from (GM) plants to bacteria takes into account the transformation of naturally competent bacteria with free plant DNA released into the soil or into the digestive tract of humans or animals (Nielsen et al., 1998). In this process, several events must occur sequentially, the likelihood of which depends on the availability of intact homologous DNA, the ability of bacteria to undergo transformation with the specific DNA, and the competitiveness of the transformed bacteria. Accordingly, the likelihood of horizontal gene transfer and incorporation of eukaryotic DNA by prokaryotes is extremely low due to genetic incompatibilities and to barriers which prokaryotes evolved to suppress this kind of gene transfer (de Vries and Wackernagel, 2005).

Evidence for horizontal gene transfer regarding recombinant plant DNA transferred to bacteria has not been obtained up to now under natural conditions. As an example, Gebhard and Smalla (1998) observed uptake and integration of transgenic plant DNA and of plasmid DNA into competent *Acinetobacter* sp. strain BD413. The authors studied the ability of *Acinetobacter* sp. strain BD413(pFG4nptII) to take up and integrate transgenic plant DNA based on homologous recombination under optimized laboratory conditions. A recombinant nptII-gene (neomycine-phosphotransferase-gene) conferring kanamycin resistance being integrated into the genome of GM sugar beet leaves was selected as marker for potential HGT. The recipient *Acinetobacter* strain was kanamycin sensitive before the transfer process. Bacteria being resistant to kanamycin when cultivated together with a homogenate of GM sugar beet leaves were taken as proof for HGT. The frequency of horizontal transfer of sugar beet DNA to *Acinetobacter* sp. strain BD413 was calculated as 1.5×10^{-10}. Tothova et al. (2010) analysed bacteria and fungi from pristine soil never exposed to glufosinate herbicide. They isolated several strains naturally tolerant to glufosinate. At the same time no *bar* gene transfer was detected under laboratory conditions to *Bacillus pumulus* either by a classical plate count method or PCR. These results indicate that HGT of *bar* genes from Bt176 maize into *B.* strains is improbable and coincidental dissemination of rare natural microbial transformants does not significantly increase the number of glufosinate-resistant soil microorganisms.

Another approach for testing HGT under optimized conditions was carried out by de Vries et al. (2004) using a specific illegitimate recombination system. Competent cells of *Acinetobacter* were exposed to plant DNA from leaf and root tissue of GM tobacco plants. The authors used the spectinomycin/streptinomycin-resistance gene as "anchor" for the transfer of segments of the tobacco plastid DNA. Horizontal transformations of genes connected to the anchor gene were found at a frequency of 1.2×10^{-7} per cell. The relatively high frequency was due to specific GC-rich microhomological sites in the *Acinetobacter* genome. These GC-rich sites may act as hotspots for stimulating illegitimate recombinations. The specific (artificial) conditions selected in the experiments (anchor-sequences plus existence of microhomological site in rector bacteria) were the prerequisites for achieving relatively high frequencies of HGT.

In considering the probability of functional gene transfer from plants into bacteria in the environment or human/animal gut, several aspects need to be taken into account (with npt II as example, see EFSA, 2007b, 2009c):

1. DNA is released from plant material by normal digestion processes that take place in the gastrointestinal tract, or by activities of nucleases present in various organisms in the environment.

2. The probability that bacteria will be exposed to DNA stretches long enough to contain the intact nptII gene is very low because of the above mentioned digestion and degradation processes (Lorenz and Wackernagel, 1994).

3. The nptII gene from plant material can only be taken up by competent bacteria via natural transformation, a process that occurs infrequently in many bacteria and in most environmental conditions (Davison, 1999).

4. If the intact nptII gene enters the bacteria, it will be rapidly degraded by restriction endonucleases in many bacterial cells which possess DNA restriction systems in order to destroy foreign DNA (Davison, 1999).

5. If the intact nptII gene does indeed survive, the probability of its incorporation into the bacterial genome is very low unless there are homologous regions already present in the bacterial genome. Gene transfer from plants to bacteria has only been demonstrated under laboratory conditions when regions of homology were already present in the recipient bacterium (Bennett et al., 2004, de Vries et al., 2001, de Vries and Wackernagel, 2002, Kay et al., 2002, Tepfer et al., 2003).

6. Expression of the incorporated nptII gene is unlikely, considering that in GM plant material the nptII gene is under the control of a promoter with preferential expression in plants, which does not support its efficient expression in bacteria.

7. Stable integration and inheritance of the nptII gene in the host bacterium is not likely in the absence of selective pressure from a relevant.

When all of the above mentioned aspects are taken into account, the probability of functional gene transfer from plants into microorganisms is extremely low. According to EFSA (2007b, 2009c) it is not surprising that transfer of an antibiotic resistance marker from GM plants to bacteria has still not been observed under natural conditions (Gay and Gillespie, 2005, Keese 2008).

Principally, the same constraints as for HGT from GM plant material to competent bacteria are effectual for incorporation of transgenes from the pollen of GM crops into the genomes of bacteria living in the gut of pollen feeding insects like bees. Mohr and Tebbe (2007) chose the following approach to detect HGT in gut bacteria: Gut bacteria were collected from the gut of bee larvae fed with pollen collected from a field cultivated with glufosinate tolerant oilseed rape. Subsequently, the gut bacteria were tested for glufosinate tolerance. 60.4% of the 96 strains tested were sensitive to glufosinate applications. The others were tolerant to differing concentrations of the herbicide. However, this surprisingly high proportion of resistant gut bacteria is not per se an indication for horizontal gene transfer. More likely, the bacterial populations already exhibited a natural resistance; therefore this approach does not seem to be appropriate for demonstrating HGT.

Several authors share the view that studies on HGT should consider specific conditions potentially enhancing HGT despite the very low likelihood of its occurrence. In addition, the appropriateness of detection methods needs attention. Nielsen et al. (1998) recommended enhancing the understanding of selection processes in the environment (e.g. in soil). Any prediction of possible consequences of the introduction of novel traits into the environment would be impossible without a thorough understanding of selection events. If specific transgenes are derived from e.g. prokaryotes or plastoms, the likelihood of HGT after degradation in soil could be higher (Monier et al., 2007). According to Nielsen and Townsend (2004), the sampling methodology and sample sizes need substantial improvement in order to ensure the collection of representative samples, reflecting e.g. the biological conditions at the sampling sites and the natural variability of microbial communities.

The structure of DNA originating from GM plants is usually not fit to allow for expression in prokaryotes (e.g. due to different promoter elements). De Vries and Wackernagel (2005) emphasized that the probability of effects of HGT to occur would increase if the potentially transferred foreign DNA conferred fitness advantage to the recipient. Nielsen et al. (2007) emphasized the importance of the acquisition of extracellular foreign DNA for the evolution of bacteria. In contrast to long strands of DNA, DNA of limited size seems to physically persist in natural media over time. The question is still not answered whether this kind of DNA is more accessible to competent bacteria than long strands of intact DNA. Heuer and Smalla (2007) addressed the necessity of checking “mobile genetic elements” (MGE), typical elements of bacteria including GM bacteria, for their potential to enhance HGT between bacterial species.

In summary, the probability for HGT from GM plants to bacteria could potentially increase if the transgene (i) conferred a specific fitness advantage, (ii) its structure was prokaryote-related and (iii) its strand length was not too long. Under these circumstances, the probability for the spread of a transgene in prokaryotes would be enhanced.

Conclusions regarding horizontal gene transfer:

- A novel GM trait may theoretically increase the frequency of horizontal gene transfer from plants to populations of microorganisms, thereby introducing new traits into microbial communities.

- However, a high degree of homology between plant DNA and bacterial genes is the prerequisite for increasing frequency of horizontal gene transfer.

- When all available information (including the origin of promoters) is taken into account the probability of functional gene transfer from plants into microorganisms is extremely low.

- Since many of the genes and the traits they express (e.g. antibiotic resistance) are prevalent in bacterial populations, HGT from GM plants will be of negligible relevance.

4.4 Impact on Target Organisms

Some GM plants produce biologically active proteins which act against the specific pests and pathogens and the target organisms are defined as the pests (eg insects, nematodes, mites) or pathogens (fungi, bacteria, viruses, protozoa,) which are targets of the genetic modification.. For example, genetically modified Cry1 Bt-plants are designed to be protected against Lepidoptera pests like the corn borer larvae and Cry3 Bt plants are designed to be protected against Coleoptera pest like the Western Corn Rootworm. The damage caused by these pests is highly variable and additional crop protection (CP) strategies are available to use with the GM plants (Dillen et al., 2010a,b).

Weeds are not considered target-organisms of a GMHT plant but are the targets of the herbicides, applied to the GMHT plant. The effects on weeds and biodiversity of the cultivation of herbicide tolerant GM plants are considered in section 4.7 effects of cultivation and management.

Genetic resistance to chemicals, and behavioural resistance to host-plant defenses and cultural practices (Onstad, 2008) such as crop rotation are known to evolve in pathogens and in insect pests, including lepidopteran and coleopteran species (Calcagno et al., 2010, Dillen et al., 2010a, EFSA, 2011c, Erasmus et al., 2010, Razze et al., 2011, Whalon et al., 2011). Resistance is also likely to develop in pests or pathogens exposed to the plant protection proteins produced by GM-crops (Andow and Zwahlen, 2006).

4.4.1 Effects on Target Pathogens

According to Tepfer (2002) and AGBIOS (2009), only a few crops with pathogen virus resistance are on the market, mainly in the USA and Canada. These GM crops are papaya with resistance against ringspot virus (PRSV), squash with resistance against cucumber mosiac virus (CMV), zucchini yellow mosaic (ZYMV) and watermelon mosaic virus (WMV); potatoes with resistance against potato virus Y (PVY) and potato leafroll virus (PLRV) and plum with resistance against plum pox virus (PPV). None of these crops are on the EU market. Lheureux et al. (2003) and Fernandez-Cornejo and Caswell (2006) reported a number of crops with resistance genes against fungal or bacterial diseases to be under development. In the EU there is considerable interest in the development of GM potatoes with resistance to potato blight. However, the authors are not aware of any report of the commercialisation of GM crops with resistance to these pathogens and so there is no information on the potential for resistance development in pathogens. Whether viral pathogens are able to develop resistant against GM-crops is subject to controversial discussion (Tepfer, 2002). However from the experience with other plant protection measures long-term resistance development cannot be excluded.

Conclusions regarding effects on target pathogens:

- The potential for pathogens to develop resistance to GM plant protection products is case by case depending on the characteristics of the pathogen and the mechanisms of resistance. However no information is currently available

- Resistance management strategies will need to be designed considering the specific characteristics of the pathogen and its intactions with the GM plant.

- It is likely that post market environmental monitoring for resistance development will be a general recommendation from EFSA for these GM crops/plants

4.4.2 Effects on Target Pests

Bt-proteins bind to specific receptors on the epithelial surface of the insect midgut, resulting in insect midgut cell bursting. Pores are formed in the membranes of the gut cells of the insect that subsequently causes cells to burst and enables midgut bacteria to enter the body cavity, which leads to septicemia and death of the larvae (Crickmore, 2005; Broderick et al., 2006, 2009; Jimenez-Juarez et al., 2007; OECD 2007, Soberón et al., 2007, 2009; Bravo and Soberón, 2008; Lemaux 2009, Raymond et al., 2010, Vachon et al., 2012). Conventional Bt preparations are considered as the most effective, specific and environmentally-friendly bioinsecticides; they have been used as biological pesticides in agriculture, forestry and in human health for the elimination of vectors of diseases for more than 60 years, but they have several limitations, such as a narrow activity spectrum, instability in rain and sunlight, and inefficiency against pest feeding on internal tissues of the plants (Sanchis, 2011). According to Sanahuja et al. (2011) conventional Bt sprays and transgenic crops have been extraordinarily successful and advantageous, with a strong record in terms of safety, efficacy, economic and environmental beneficence.

The vast majority of insect resistant (IR) GM plants (maize, cotton, potato, tomato) placed on the market express Cry-Proteins against lepidopteran pests (Cry1Ab, Cry1Ac, Cry1F, Cry2Ab, Cry9C1) or coleopteran pests (Cry 3A, Cry 3Bb1, Cry34Ab1, Cry35Ab1) (OECD, 2007, AGBIOS, 2009). Maize varieties expressing various combinations of Cry 1 and Cry 3 are now being developed and marketed in USA and elsewhere to provide protection against both classes of pests. At the end of 2007, only MON810 maize expressing Cry1Ab was available on the market for cultivation purposes in Europe. However, Ranjekar et al. (2003) reported that other IR-crops have been developed expressing proteins against target pests like proteinase inhibitors, amylase inbibitors, chitin degrading enzymes or plant lectins. In addition synthetic Cry proteins in various crop plants were reported to be under investigation (Ferré and van Rie, 2002). There is no data on potential resistance development in target pests to non-Bt systems in IR GM. For the EU, the literature review is currently of relevance only for GM crops expressing Bt-protein.

The toxic pathway involves (i) ingestion of Cry protein by susceptible insects, (ii) solubilization of crystals, (iii) release of protoxins, (iv) processing of protoxins by midgut proteases into a protease-resistant core fragment (the toxin), (v) passing of the toxin through the peritrophic membrane, (vi) binding to a specific receptor located on the brush border of the midgut cells, (vii) partial insertion of the toxin into the membrane, (viii) pore formation, (ix) cell lysis followed eventually by cell death (Schnepf et al., 1998).

McGaughey (1985) published the first report on Bt-resistance development in 1985. The Indian meal moth from grain bins developed a 100-fold level of resistance against conventional Bt-insecticides after laboratory selection. Ferré and van Rie (2002) gave a broad overview of further Bt resistant pest strains evolving during the following decades, specifically for several lepidopteran pests. Here, the first case of field resistance was observed in Hawaii. Populations of the diamondback moth *Plutella xylostella* showed a reduced susceptibility to Bt-sprays (Tabashnik et al.,1990). A decreasing susceptibility and increasing resistance or increasing frequency of resistance genes against Bt-protein expressed in GM cotton was observed for lepidopteran pests in the field several years after cultivation in Australia, India, USA, and China (Zhao et al., 1996, 2000, Shen et al., 1998, Fakhrudin et al., 2003, Gunning et al., 2005, Downes et al., 2007, Bagla, 2010, Dhurua and Gujar, 2011, Tabashnik et al., 2012).

Field resistance is defined as a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field (Tabashnik, 1994; Andow, 2008). Instances of field resistance to Bt-maize have been reported outside Europe for two lepidopteran target pests in maize that are not present in the European fauna (Tabashnik et al., 2009; Huang et al., 2011b): *Busseola fusca* (Van Rensburg, 2007; Kruger et al., 2009, 2011b) and *Spodoptera frugiperda* (Matten et al., 2008; Moar et al., 2008; Tabashnik, 2008; Tabashnik et al., 2008a; Storer et al., 2010). The field resistance in a population of the African stem borer (*B. fusca*) in South Africa, where some larvae were able to survive on Cry1Ab-expressing maize (Van Rensburg, 2007; Kruger et al., 2009a, 2011b) resulted from a combination of a late general planting date with consequent increased levels of infestation and variance in time of planting, providing a continuous supply of moths (Kruger et al., 2009a). The recent survey by Kruger et al. (2011a) revealed that compliance with refugia requirements in the region was low especially during the initial 5-7 years after release and a high number of farmers applied insecticides as preventative sprays on Bt-maize and refugia irrespective of stem borer infestation levels.

Another instance of pest resistance concerns fall armyworm, *S. frugiperda* (for a review see Storer et al., 2012). Larvae surviving on Cry1F-expressing maize in some fields on an isolated tropical island in the USA (Puerto Rico) were collected and exposed to high concentrations of the Cry1F protein in laboratory bioassays, where no mortality was observed (Matten et al., 2008; Moar et al., 2008; Tabashnik, 2008; Tabashnik et al., 2008a). Recently, Storer et al. (2010) confirmed via laboratory bioassays that *S. frugiperda* collected from the affected area exhibited lower sensitivity to the Bt protein compared with typical colonies from other regions, and that the resistance was shown to be autosomally inherited and highly recessive. The unusual combination of biological, geographic, and operational factors (such as high selection pressure for resistance by continuous silage maize production with sequential year-round plantings, high level of overall *S. frugiperda* pest pressure during the year of observing its damage on Bt expressing hybrids, drought conditions reducing availability of alternative host plants that encouraged movement of the adult and larval populations into irrigated agricultural maize fields led to *S. frugiperda* evolving resistance to the Bt protein in Puerto Rico. Moreover, no insect resistance management (IRM) measures were put in place at that time in Puerto Rico. It is considered very unlikely that the levels of selection pressure exerted on *S. frugiperda* by Bt maize cultivations in Puerto Rico will be experienced in the EU, with the exception of overseas territories. *S. frugiperda* is a migratory seasonal pest across most of the USA, cannot develop at temperatures below 12°C, and displays one or two generations per year in the USA. This reduced selection pressure on *S. frugiperda*, the availability of alternative host-plants and the implementation of IRM plans make the evolution of resistance as observed in Puerto Rico unlikely in other regions (EFSA, 2011c). In the future, pyramided trait products that produce two or more different Bt proteins are being introduced to further delay resistance development to Cry1F (Storer et al., 2012).

Resistance development of pests in the field has so far not been reported in other GM Cry1 Bt-plants (EFSA, 2011c). In particular, in different Bt-maize cultivars resistant to lepidopteran or coleopteran pests no resistance development in the field has been observed either in the USA or in Europe (Farinós et al., 2004, Eizaguirre et al., 2006, Siegfried et al., 2005, Stodola et al., 2006, Huang et al., 2007). However European Corn Borer populations with a decreased susceptibility to Bt-proteins were selected in the laboratory by Huang et al. (1997), Bolin et al. (1999), Chaufaux et al. (2001) and Alves et al. (2006). Considering that European climatic and maize cultivation conditions differ from those observed in Puerto Rico and South Africa, EFSA (2011c) concluded that field resistance would not develop rapidly in EU target pests such as the European corn borer (*Ostrinia nubilalis*) and the Mediterranean corn borer (*Sesamia nonagrioides*).

From the breeders point of view the potential development of resistence of target organisms is a serious problem since their products would be useless in specific regions. To delay evolution of pest resistance breeders introduced multiple (stacked) Cry toxins into maize lines. The strategy relies on the assumption that simultaneous evolution of resistance to slightly differently acting cry-proteins would be highly unlikely. An example is the transformation of specific Bt-cotton lines with Cry1Ac and Cry2Aa genes for improving long-term effects on *Heliothis virescens* (Tobacco budworm; Jurat-Fuentes et al., 2003). Another strategy for avoiding evolution of resistance in pest insects is the construction of synthetic

Cry-genes whose proteins are equipped with multiple binding domains. An example is the synthetic Cry1.105. It consists at least of binding domains of Cry1Ab, Cry1Ac and Cry1F. All of them are directed against lepidopteran larvae (MON 89034 http://www.efsa.europa.eu/EFSA/DocumentSet/gmo_02_partii_summary,0.pdf).

Summarizing the available literature, resistance development of lepidopteran species against Bt-protein has not been observed in Europe (as at end 2011); this also holds true for Bt-maize cultivation worldwide with the exception of South Africa and Puerto Rico. So far, the applied insect resistance management seems to be a successful measure for delaying or preventing resistance development during several years of large scale cultivation (Tabashnik et al., 2003, Bates et al., 2005). Additionally knowledge about the potential for resistance development has been gained due to extended studies about basal susceptibility and the frequency of resistance alleles in Europe (e.g. Chaufaux et al., 2001, Ferré and van Rie, 2002, Bourguet, 2004, Saeglitz et al., 2006, Schuphan, 2006, Gaspers et al., 2010). However, several of these authors regard resistance development for Bt crops to be a question of time.

Transgenic Cry1 proteins are pore-forming toxins producing ion channels in lipid membranes of gut of targeted lepidopteran pests (Rausell et al., 2004a,b, Bravo et al., 2007, Gomez et al., 2007, Pigott and Ellar, 2007). Because insect pests have been able to develop resistance to chemical insecticides applied to control them (Whalon et al., 2008), the potential development of insect resistance to Cry proteins constitutively expressed in GM crops is considered as a relevant concern in the ERA (e.g. EFSA, 2008b,c, Gassmann et al., 2009). Resistance development generally refers to a genetically-based decrease in a population's susceptibility to a toxin and can be evaluated with laboratory bioassays estimating the resistance ratio, which is the LC50 (concentration of toxin killing 50% of the larvae) of a field-derived strain divided by the LC50 of the susceptible strain (Saeglitz et al., 2006; Andow, 2008; Bravo and Soberón, 2008). Susceptibility is usually measured by sampling insects from a field population and determining how their progeny respond to the toxin in laboratory experiments (Tabashnik et al., 2008a, Huang et al., 2011a).

Major lepidopteran target pests of the Cry1Ac expressing cotton and Cry1Ab expressing maize (such as MON810) have been monitored worldwide for potential resistance development against specific Cry1 proteins. A recent meta-analysis of available monitoring data indicated that neither in the EU, nor in the United States (US), have populations of resistant European and Mediterranean corn borer been found in regions where Cry1Ab expressing maize is grown (Tabashnik et al., 2008a), confirming previous observations (Andow et al., 2000; Bourguet et al., 2003; Farinós et al., 2004; Eizaguirre et al., 2006; Schuphan, 2006; Stodola et al., 2006; Andreadis et al., 2007). In Spain, for instance, after many years of field exposure of corn borer populations to Cry1Ab expressing maize, no indications of resistance development were found (Farinós et al., 2004; Eizaguirre et al., 2006; Andreadis et al., 2007, Farinós et al., 2011). So far, F_2 screenings (Andow and Alstad, 1998) performed on mated females collected from the field in Mediterranean EU countries and their progeny reared under confined conditions, failed to detect major resistance alleles in corn borer populations (Bourguet et al., 2003; Schuphan, 2006; Andreadis et al., 2007). These data indicate that dominant resistance alleles are extremely rare in populations of corn borers and also that the initial frequency of recessive resistance alleles is low (Andow et al., 1998, 2000; Bourguet et al., 2003; Schuphan, 2006; Stodola et al., 2006; Andreadis et al., 2007). In contrast, laboratory selections for resistance with Cry1Ab toxins have yielded partial resistance levels in some corn borer strains after many generations (Chaufaux et al., 2001; Huang et al., 2002; Farinós et al., 2004; Alves et al., 2006; Schuphan, 2006). While resistance levels fluctuated between generations for each strain, toxin susceptibility decreased significantly over generations for all selected strains. However, none of the laboratory-selected resistant corn borer larvae studied by Farinós et al. (2004) survived on Bt-maize seedlings. It is thus questionable whether these levels of resistance will reflect potential resistance development upon exposure of field populations to Bt-crops (e.g., Bourguet, 2004). Moreover, even though partial resistance has been shown to be reasonably common in some European corn borer populations (Bourguet et al., 2003), the polygenic nature of resistance in tested laboratory strains suggests that major genes for resistance to the Cry1Ab protein are rare in founding populations of the European corn borer (Alves et al., 2006).

Available data indicate that recessive resistance alleles are rare in populations of European and Mediterranean corn borers. Moreover, according to the EU research project ProBenBt in which various aspects of European and Mediterranean corn borer genetics and Cry1 resistance in targeted lepidopteran pest species were studied, gene flow among European populations of both pest species is likely to be high enough to delay resistance development to Cry1 proteins in maize (Schuphan, 2006). The fact that some adults of the European corn borer mate at a more restricted spatial scale (Hunt et al., 2001; Qureshi et al., 2005; Dalecky et al., 2006; Bailey et al., 2007) than previously assumed in the high-dose/refuge strategy might under certain circumstances (e.g., crop rotated landscape) decrease its efficiency (Dalecky et al., 2006; Schuphan, 2006), as well as the infestation rate of the pest with a natural parasite (Lopez et al., 2010). However, predictions generated by a recently developed demo-genetic dynamic model confirm that applying the high-dose/refuge resistance management strategy is likely to maintain the sensitivity to Cry proteins in pest species (Tyutyunov et al., 2008, Liu et al., 2011). An alternative strategy is the refuge in a bag approach where GM and non-GM seeds are mixed and sown together so that the crop contains a proportion of non-GM plants and the selection pressure on the pest population is reduced. Generally this is not considered as effective as other refuge strategies (Murphy et al., 2010, EFSA, 2012b).

In areas where resistance is found to be developing or where few or no refugia are established, the release of sterile insects to mate with resistant insects can be used to prevent the build up of resistant populations (Tabashnik et al., 2010, Wu, 2010).

To delay or prevent the potential development of insect resistance to Bt-crops, a resistance management tactic, relying on a ‘high dose/refuge strategy’, has been endorsed in the US and EU (Bates et al., 2005, Andow, 2008, Bravo and Soberón, 2008, Gassmann et al., 2009, Andow et al., 2010). The high dose/refuge strategy intends to reduce the selection pressure for resistance alleles by combining Bt-maize that produces a high dose of toxin with non-Bt-maize plants that are grown nearby as a refuge (Ives and Andow, 2002). To ensure that individuals heterozygous for a resistance allele are killed by the Cry1Ab protein produced in plant tissues, the increase in fitness conferred by resistance alleles must be recessive. The study of fitness costs associated with resistance to Cry proteins is important for understanding resistance evolution and for evaluating resistance management practices (Crespo et al., 2010, Pereira et al., 2011). The second assumption of the high dose/refuge strategy is that resistance alleles must be rare, so that only few homozygotes survive on Bt-crops. Finally, it is assumed that the few resistant insects emerging in Bt-crops must mate randomly or preferentially with the larger pool of susceptible insects preserved on non-Bt-crops (Alstad and Andow, 1995; Andow, 2008). Mating pattern between susceptible and resistant insects need to be considered (Blanco et al., 2010) as well as the design of reguges (Onstad et al., 2011). The implementation of the ‘high-dose ⁄ refuge’ IRM strategy has been successful during the last 15 years in substantially delaying field resistance to Bt crops (Huang et al., 2011b). Moreover, Bt maize has become so widely adopted in U.S. agriculture that Hutchison et al. (2010) found areawide suppression of the primary pest (European Corn Borer) benefitting also farmers using conventional maize.

Another strategy that is being developed is to stack genes expressing different cry toxins that are effective against the same target pest. For example Cr1Ab and Cry1F are combined to give control of lepidopteran pests while reducing the likelihood of resistance developing to each toxin.

The cultivation of Cry1 expressing maize in the EU is currently on a small scale and limited to a few geographic regions. In addition, as potential resistance development is dependent upon multiple factors, predicting future responses of corn borer populations in Europe is case specific (Tyutyunov et al., 2008). Dispersal distances, for instance, have been shown to be influenced by plant size, weather conditions during the flight, pheromonal patterns in the field and the timing of the flight (Hunt et al., 2001; Engels et al., 2008). Therefore, the potential development of resistance in target pests should be monitored in order to detect potential changes in resistance levels in pest populations. Applicants are generally requested to monitor resistance development in target pests in the US and Canada. In this respect Downes et al. (2010) report of field evolved resistance to a protein in a dual-toxin Bt-crop in Australia. This observation has precisely fulfilled the intended function of monitoring for resistance. Such monitoring provides an early warning of increases in frequencies that may lead to potential failures of the transgenic technology. Furthermore, Downes et al. (2010) demonstrate that pyramids so more than one Bt protein does not exclude resistance development.

In the EU, the monitoring of Cry1 expressing maize demands case-specific insect resistance management and considers further general surveillance through farmer questionnaires (Schmidt et al., 2008).

The possible evolution of resistance to Bt protein in lepidopteran target pests is considered by EFSA as a relevant environmental and agronomic concern associated with the cultivation of Bt maize, as the consequences of resistance evolution may lead to altered pest control practices that may cause adverse environmental effects. However, appropriate risk mitigation measures and monitoring can address the risk proportionately (see e.g. EFSA, 2011c).

In the case of coleopteran related pests, e.g. the Western corn rootworm, the possible evolution of resistance to the Cry3Bb1 protein has been demonstrated for maize MON 863 under artificial selection experiments under greenhouse conditions (Meihls et al., 2008) and recently confirmed under field conditions in some populations in Iowa, USA (Gassmann et al., 2011).

- Meihls et al. (2008) exposed Western Corn Rootworm (coleopteran) colonies to Bt-maize in the greenhouse under four selection regimes (1) continuous exposure (larvae were reared on Bt-maize throughout the larval development period), (2) neonate exposure (larvae were placed on Bt-maize as neonates, then shifted to non-Bt-maize to complete development), (3) late exposure (larvae ate non-Bt-maize as neonates and completed development on Bt-maize), and (4) no exposure (larvae were reared on non-Bt-maize). After three and six generations of greenhouse selection, the colony that was continuously exposed to Bt-maize was highly resistant; larval survival on Bt-maize was equivalent to survival on the non-Bt-maize counterpart. After three generations of selection, the LC50 of the continuous exposure colony was approximately 22-fold greater than that of the unexposed control colony. After six generations of selection, percent survival on Bt-maize relative to its non-Bt-maize counterpart was 11.7-fold greater in the field for the continuous exposure colony than for the control colony (Meihls et al., 2008).

- Gassmann et al. (2011) reported that the survival of Western corn rootworm on Cry3Bb1-expressing maize in laboratory bioassays was significantly higher for individuals from problem fields where farmers reported severe root injury to Cry3Bb1-expressing maize than from control fields where such injury was not reported. In all problem fields studied, Cry3Bb1-expressing maize had been grown for at least three consecutive years (Gassmann et al., 2011), corresponding to three generations of selection (Gray et al., 2009).

These results demonstrate that Western corn rootworm will rapidly evolve resistance to Cry3Bb1-expressing maize under conditions of continuous exposure (Meihls et al., 2008; Tabashnik, 2008; EPA, 2010; Hibbard et al., 2010, Gassmann et al., 2011; Oswald et al., 2011; see also Lefko et al., 2008 and Nowatzki et al., 2008 for the Cry34Ab1/Cry35Ab1-expressing maize event DAS-59122-7, and Meihls et al., 2011 for the mCry3A-expressing maize event MIR604). It is evident that these maize types do not meet the functional definitions of high dose, so that the utility of refuge, refuge size and refuge placement for delaying rootworm resistance should be further investigated (Binning et al., 2010).

Based on risk assessments, the EFSA GMO Panel advocates the deployment of diversified resistance management strategies, along with more integrated methods to control coleopteran pests targeted by Bt-crops (e.g. EFSA, 2011d).

It should be noted that the effectiveness of the control of a target insect pest may lead to the emergence of secondary pests. In China, the control of *H. armigera* by Bt cotton cultivation has resulted in a decrease in the amount of insecticides used on Bt cotton compared to conventional cotton. This has led to a lack of control of mirids on Bt cotton due to the reduction in broad-spectrum insecticide use and consequently to a transformation of a minor pest to a main one. Therefore, it is important to develop pest control using a broad spectrum of practices. These include crop rotation, tillage, high biological diversity in the farming system and promotion of the pests' natural antagonists (Berge and Ricroch, 2010). According to a review by EFSA (2011c) arthropod assemblages in agricultural fields are in a continuous fluctuation in terms of their species number, composition and individual densities over time and space. Human interventions, including pest control, also influence these parameters. Whenever pest management of crops changes, the abundance of some pest species may decline and other pest species may increase. Reduced or no insecticide applications in Bt crops (e.g. maize and cotton), that express selective Cry proteins, provide an opportunity for secondary pest species, previously controlled by insecticides used against key target pests, to reach damaging levels. Natural enemies failing to fully control secondary pests, and reduced competition with target pests might also play a role in secondary pest outbreaks (Catangui and Berg, 2006, Sanvido et al., 2007, 2008a, Eichenseer et al., 2008, Romeis et al., 2008b, Fitt, 2008, Kennedy, 2008, Naranjo et al., 2008, Dorhout and Rice, 2010, Lu et al., 2010, Virla et al., 2010, Hutchison et al., 2011). However, the emergence of secondary pests is not specific to the cultivation of GM crops but a common phenomenon in a range of integrated agricultural managment systems (see e.g. the critical discussion of Then, 2010 by Hutchison et al., 2011).

In an integrated pest management (IPM) context, Bt maize can be regarded as a preventive (host plant resistance) or a responsive pest control measure (Meissle et al., 2011).

Conclusions regarding effects on target pests:

- Bt proteins can cause resistance development in target pests, which results in a loss of environmentally desirable plant protection tools.

- Resistance development in target insect pests is most likely to occur in regions where Bt crops are deployed most extensively for several years and selection pressure is highest. Due to the nature of cropping in Switzerland it is very unlikely that this will be a focus for resistance development.

- Post market environmental monitoring for resistance development is a general recommendation (from EFSA, 2011c,d) for all Bt crops.

- Resistance development of target organisms can be delayed by a range of management techniques e.g. as described in EFSA (2011c,d)

- Bt maize is a highly specific tool that efficiently controls the main pests and allows combination with other preventive or responsive measures to solve other agricultural problems including those with secondary pests (Meissle et al., 2011).

4.5 Impact on NTOs

The potential impact on non-target organisms is addressed in various international documents where it forms a substantial part of any environmental risk assessment of GMOs (e.g. EC, 2001, EC, 2002, Canadian Food Inspection Agency, 2004, EFSA, 2006a, Rose, 2007).

An assessment is required of the possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GM plant with non-target organisms, including the impact on population levels of competitors, herbivores, symbionts (where applicable), predators, parasites and pathogens (EFSA, 2006). Several detailed descriptions for NTO risk assessment procedures, including selection criteria for the NTO test species and test methods that can apply to different regions, are provided in guidance documents which can be adapted for specific crops in specific agriculture ecosystems (EFSA, 2010a, 2010b, Romeis et al., 2009). Tests on non-target organisms covering bi- and tri-trophic interactions including direct and indirect effects are widely accepted in risk assessment and results are widely published in the literature (BEETLE, 2009).

A number of review papers particularly emphasize the effects of GM plants producing insecticidal proteins on non-target arthropods (Andow and Hilbeck, 2004, Hails and Raymond, 2004, Dolezel et al., 2005, Lövei and Arpaia, 2005, O'Callaghan et al., 2005, Andow and Zwahlen, 2006, Hilbeck and Schmidt, 2006, Schuler, 2006, Sanvido et al., 2007, Greenpeace, 2007, Marvier et al., 2007, Widmer, 2007, Woiwod and Schuler, 2007, Romeis et al., 2007, Farinós et al., 2008, Arpaia, 2010, Bartsch et al., 2010, EPA, 2010, Romeis et al., 2010, Marvier, 2011, Meissle, et al., 2011).

An extensive body of research data has been assembled on non-target impacts of maize expressing insecticidal Bt proteins, like the varieties MON810, Bt176, Bt11, and maize 1507. One important lesson according to Schuler (2006) is that negative effects observed in the laboratory do not necessarily translate into impacts in the field where many other factors affect the impact on non-target species (including climate, food availability and predation). The majority of studies reviewed by Schuler (2006) do not show any unexpected negative effects on non-target insects.

A tiered process of toxicity testing is generally used to assess the non-target effects posed by traditional insecticides because it is suitable for assisting the decision-making process in an effective and rigorous way (Romeis et al., 2008a). The application of tiered approaches is widely accepted (Rose, 2007, EFSA, 2006), but differences appear e.g. how to use the results for decision making. There is considerable disagreement about the most appropriate framework for using ecological approaches (Andow et al., 2006a,b, Andow and Zwahlen, 2006; Romeis, 2006, Romeis et al., 2006a), and a major difference between the approaches is related to confidence and certainty in decision making within the tiered framework (EFSA, 2008, BEETLE, 2009). It is clear that ecotoxicological methods developed for the testing of pesticides are not always suitable for risk assessment of GMPs (Römbke et al., 2010, Romeis et al., 2010). However, the ERA framework provided by EFSA (EFSA, 2010a,b) makes use of the benefits of the various approaches.

Based on a three year interdisciplinary research project called ECOGEN, Birch et al. (2007) provided a detailed discussion of the role of laboratory, greenhouse and field scale experiments in understanding the interactions between GM plants and soil ecosystems. They concluded that results were not predictive between the three experimental scales, but have value when used with feedback loops between the scales, which can be used to address questions raised by results from any level of experimentation and also for putting GM crop risks into context with current agricultural practices in regionally differing agro-ecosystems.

Another review was published by Andow and Zwahlen (2006), which provides an overview on ERA development since the end of the 1980s. The authors assessed previous models for risk assessment and a conclusion was that future genetically modified crops are likely to pose greater challenges for risk assessment, and meeting these challenges will be crucial in developing a scientifically coherent risk assessment framework. However, scientific understanding of the factors affecting environmental risks to NTOs is an ongoing task where environmental scientists need to help improve environmental risk assessment methods.

It is helpful for the assessment to group the published literature into categories based on the tiered approach to testing (see below). The BEETLE report (BEETLE 2009) suggests a grouping into the following categories:

0 Literature reviews or modelling approaches

1 Tier 1 Laboratory studies with purified insecticidal protein

2 Tier 1 and 2 Laboratory or glasshouse studies with GM insecticidal plants
(or parts of plants)

3 Tier 3 Semi-field studies (contained environment) with GM insecticidal plants

4 Tier 4 Field studies with GM insecticidal plants (open environment)

For the purpose of NFP59, we conclude that ‘tiering’ provides an internationally accepted concept and recommend making use of the tiered approach developed by the international initiative of Romeis et al. (2008a). This approach focuses on the formulation and testing of clearly stated risk hypotheses, making maximum use of available data and using formal decision guidelines to progress between testing tiers (see Figure 3). This tiered approach is intended to provide guidance to regulatory agencies that are currently developing their own NTO risk assessment guidelines for GM crops and to help harmonize regulatory requirements between different countries and different regions of the world.

The following sub-chapters on potential effects of GMO on NTO reiterates the categories developed by the BEETLE (2009) report.

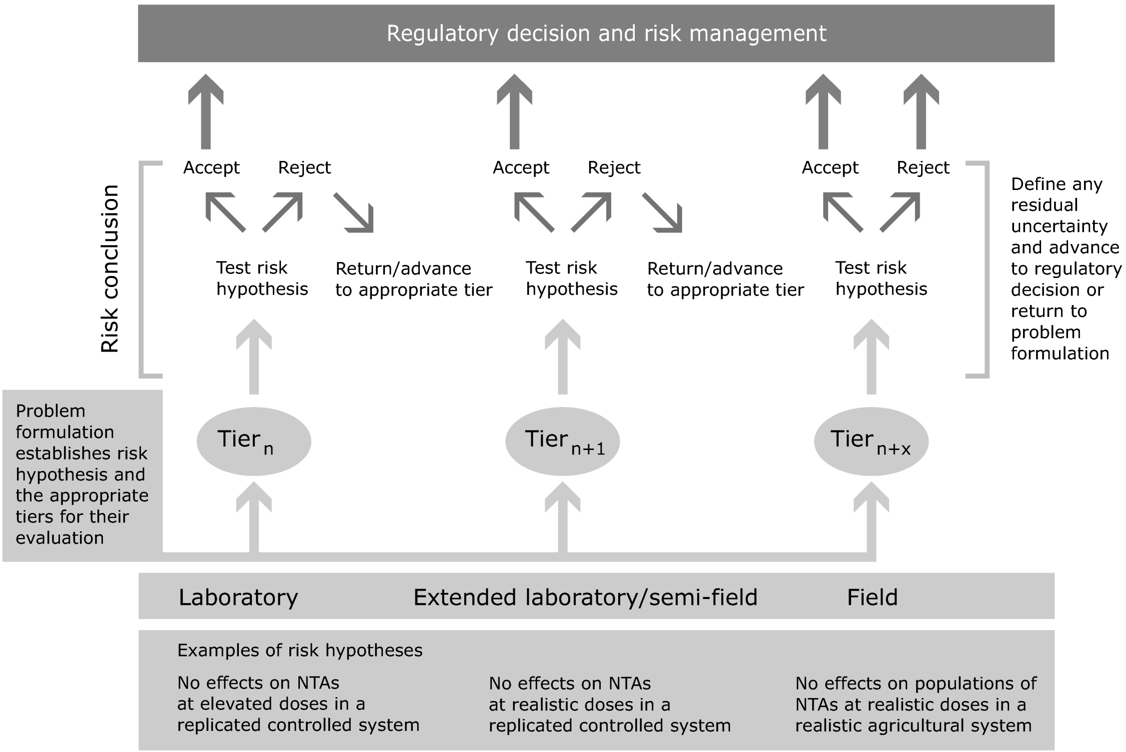


Figure 3: Tiered approach according to Romeis et al. (2008a).

4.5.1 Direct Toxic Effects on Plant-Associated NTOs

Genetically modified crops that express insecticidal Cry proteins derived from the soil bacterium *Bacillus thuringiensis* (Bt) have been cultivated since 1996 (Clive, 2008). Bt is an insecticidal bacterium that has successfully been used as a biopesticide for many years. According to Raymond et al. (2010) the bacterium *B. thuringiensis* is a true pathogen that reproduces in an insect cadaver. Bt spores are prevalent in the environment and it can act as an opportunistic pathogen under appropriate conditions. In their critical review, Vachon et al. (2012) concluded that the presently available information still supports the notion that Bt Cry proteins act by forming pores, but most physiological events leading to their formation, following binding of the activated toxins to their receptors, remain relatively poorly understood.

Each Cry protein is highly specific to species groups like lepidoptera and coleoptera and are targeted at pests of these groups (O'Callaghan et al., 2005). GM Bt plants can express the specific Cry proteins in a range plant organs (including pollen, anthers and roots) depending on the specific GM-event. As a consequence larvae of target species (e.g. European Corn Borer (ECB) or Western Corn Rootworm (WCR)) as well as larvae of non-target species (e.g. butterflies or beetles) are likely to be exposed. Lepidopteran and coleopteran specific Cry proteins have only been used in maize until now in the EU, and only one Cry1Ab expressing maize (MON810) is cultivated so far.

It is well known that a range of lepidopteran species larvae could potentially be affected by Cry1 proteins (Losey et al.,1999, Felke and Langenbruch, 2002, 2005, Lang and Vojtech, 2006, Prasifka et al., 2007, Felke et al., 2010). Data on effects are mainly derived from laboratory tests but some are derived from maize crops (for a review see Evans, 2002). However, exposure of any populations of NTO larvae to the toxin is restricted to those consuming the Bt plant or its products. Such NTO larvae may be exposed to the Cry protein when Bt maize pollen is deposited on plants on which they are feeding in the fields or margins of Bt maize fields. This was confirmed by the extensive semi-field and field experiments conducted in USA and Europe (Oberhauser et al., 2001, Pleasants et al., 2001, Sears et al., 2001a,b, Stanley-Horn et al., 2001, Wright et al., 2000, Dively et al., 2004, Lang, 2004, Gathmann et al., 2006a,b). However, in all studies no or minor effects on non-target lepidopteran species and populations were reported for maize varieties that express low levels of Cry1Ab protein in pollen.

Anthers have a higher concentration of Cry1Ab protein than pollen and in a similar level to that in maize leaves (e.g. in MON810 maize, between 0.30-6.65 µg/g as reported in Nguyen and Jehle, 2007). In laboratory experiments the consumption of anther fragments of maize MON810 caused a significant increase in mortality in monarch butterfly larvae (Felke and Langenbruch, 2005), confirming previous observations made by Hellmich et al. (2001), Anderson et al. (2005), Prasifka et al. (2007). However, anthers are not considered to contribute significantly to European lepidopteran larvae exposure to Cry1Ab since anthers show a much lower spatial and temporal distribution compared to pollen (EFSA, 2009a).

In the course of the evaluation of three applications for renewal of authorisation maize MON 810 (EFSA, 2009a) the EFSA GMO Panel used a new risk assessment methodology, published later by Perry et al. (2010), in order to simulate and assess potential adverse effects on non-target Lepidoptera after ingestion of harmful amounts of Cry1Ab-containing maize pollen deposited on their host-plants. On the basis of the data provided by the applicant and obtained from a literature survey and a modelling exercise, EFSA concluded that the amounts of maize MON 810 pollen grains found in and around maize fields are unlikely to adversely affect a significant proportion of non-target lepidopteran larvae. The EFSA Panel also noted that all modelling exercises are subject to uncertainties and, as with any ecological model, further data would refine the estimates reported. Hence, the EFSA GMO Panel considered it advisable that, in areas with sensitive non-target Lepidoptera present, higher levels of adoption of the cultivation of maize MON 810 be accompanied by management measures in order to mitigate the possible exposure of these species to maize MON 810 pollen (EFSA, 2009b).

Bt-maize pollen might be hazardous to a range of lepidopteran species of conservation concern (Lang and Otto, 2010), and should therefore be the focus of specific risk management (Hofmann et al., 2010, 2011). The mathematical model, developed for maize MON 810, was recalibrated and extended to estimate the effects of maize 1507 pollen on non-target Lepidoptera and the efficacy of certain mitigation measures (Perry et al., 2011, EFSA, 2011c,d). In situations when the proportion of maize and uptake of maize 1507 are sufficiently high, then highly sensitive non-target Lepidoptera populations might be at risk, and the EFSA GMO Panel recommends that mitigation measures are adopted to reduce exposure. Risk managers are provided with tools to estimate global and, where needed local, mortality of exposed non-target Lepidoptera, both before and after different mitigation measures are put in place, and for different host-plant densities. Mitigation measures are only needed when the proportion of maize and uptake of maize 1507 are sufficiently high, and when highly sensitive Lepidoptera species are present. If maize 1507 cultivation remains below 5% of the Agricultural Unit of Account[8], then risk mitigation measures are not required. EFSA concluded that, subject to appropriate management measures, maize 1507 cultivation is unlikely to raise safety concerns for the environment (EFSA2011c,d).

There was some discussion about the usefulness of such modeling excercises in the light of incomeplete data and thus remaining uncertainties. Lang et al.'s (2010) main objections to

---

[8] For example, an uptake of 20% of maize 1507 in a region where maize represents 25% of the arable land.

quantitative conclusions from such models for the ERA of GM plants was the need to allow for the fact that sensitivity to a Cry protein can vary greatly among lepidopteran species, even within genera, making a prediction of the sensitivity to a Cry protein for any given species difficult. As Perry et al. (2010, 2011) confirmed, extrapolating observations made on one non-target lepidopteran species to another is problematic because of between-species variability in acute sensitivity to Cry1 proteins (see also Schmitz et al., 2003). EFSA (2011b) has addressed this problem by providing output from the model for a range of non-target lepidopteran species with different sensitivities. Risk managers will need to assess which species may be at risk, and modulate management options according to any known or assumed sensitivities of these species.

Kjaer et al. (2010) examined the consequences of Cry1Ac expressing GM oilseed rape for larvae of *Pieris brassicae.* Based on laboratory feeding studies and modeling the authors concluded that under certain circumstances the ecological consequences can be severe for susceptible butterfly populations. Such models may both aid the design of further tests for effects and support the assessment whether population effects are likely to occur due to the presence of insect-resistant plants outside the agricultural area.

Surveys of biota in the main Bt maize growing areas of Spain (Eizaguirre et al., 2006, Perry et al., 2010) have revealed an absence of wild lepidoptera occurring during the flowering period of maize and thus there is no exposure in this and some other areas of Europe. Exposure and hence risks to sensitive Lepidoptera are considered to be generally very low across the maize growing regions of Europe and only likely to occur in a few situations. This contrast with the use of insecticides in maize which are applied earlier in the season and so larger numbers of Lepidoptera are likely to be exposed.

Potential detrimental effects on NT coleopteran species have been studied as these could be expected from crops expressing coleopteran specific cry-proteins e.g. Cry3Bb1. Beetle species feeding on plant parts as well as carnivorous species such as rove beetles or carabid beetles are exposed to the Cry protein (Harwood et al., 2005, 2006, Zwahlen and Andow, 2005, Obrist et al., 2006a,b,c). Tier 1 lab studies showed increased mortality or sublethal effects on longevity of ladybird or carabid beetles (Bai et al., 2005, Meissle et al., 2005, Schmidt et al., 2004) under worst-case high exposure/intake situations.

Of particular interest is the study of Schmidt et al., 2009 (see also Schmidt et al., 2004), which was used by some EU Member states to defend a temporary ban on MON810 Bt maize cultivation. Schmidt et al. (2009) carried out laboratory toxicity tests with microbially produced trypsin-activated Cry1Ab or Cry3Bb proteins applied to different larval stages (L1-L4) of the coccinelid *Adalia bipunctata*. Bt-protein treatment was performed via 0, 5, 25, or 50 µg/ml Bt-protein spray on Ephestia sp. eggs, which were then offered as food in a no-choice test. The authors did not quantify the actual intake of Bt-protein by the larvae, but tested qualitatively the presence of the Bt-proteins in the spray solution by immuno-strip assays. The paper reports that *A. bipunctata* larvae fed lepidopteran-active Cry1Ab protein at the lowest concentration (5 µg/ml) of the protein as well as the higher levels exhibited significantly higher mortality than the control group. However, in experiments with the coleopteran-active Cry3Bb toxin, only a concentration of 25 µg/ml resulted in a significantly higher mortality compared to the control. Both experiments revealed a slight decline in mortality at the highest concentration of 50 µg/ml, though this was statistically significant only in the case of Cry1Ab treatment. No differences were detected for development time of larvae and body mass of newly emerged adults. The authors re-iterated (Hilbeck et al., 2012a,b) that the increased mortality of larvae in the toxin feeding trials was caused directly by the activated Cry-proteins and raised questions regarding their suggested postulated specificity and their mode of action in *A. bipunctata*. The authors also reiterated that their lower tier laboratory studies including the Schmidt et al. (2009) studies "*provide indications for possible hazards that require further investigation (or possibly long-term field monitoring) to determine whether they pose a risk or translate into 'harm' in the field: no more and no less.*"

However, neither a dose-response relationship, nor sublethal effects (on developmental time and adult body weight) on surviving specimens were observed; both these features represent a typical response of sensitivity to Cry proteins. The higher toxicity of a Lepidoptera-specific Cry1Ab reported on Coleoptera in comparison to the more Coleoptera-specific Cry3Bb is an outcome that needs to be confirmed based on more quantitative data (both on food intake and actual protein concentration). However these data are not sufficient to identify a hazard or indicate a new mode of action of Cry proteins on the coccinellid species tested. The experimental design has some obvious weaknesses (e.g. no measurement of the actual intake of Cry protein as recommended by Meissle and Romeis, 2009). Romeis et al. (2012) do not see verified evidence that *A. bipunctata* larvae are sensitive to Cry1Ab at realistic worst-case exposure concentrations. This, together with the fact that A. bipunctata will be little exposed to Cry1Ab under field conditions, allowed Romeis et al. (2012) to conclude that the risk of Bt maize to this predator is negligible. Support for this comclusion comes from the results of many Bt maize field studies that have not revealed evidence for direct Cry1Ab effects on non-Lepidoptera species (e.g. as referenced in EFSA, 2012d).

Garcia et al. (2010) found no effect of Cry1Ab on the rove beetle *Atheta coriaria* exposed through MON810 maize fed-prey. MON810 maize did not influence the overall community structure of rove beetle (Staphylinidae) according to a three year study from 2001-2003 in Hungary (Balog et al., 2010). After grouping staphylinids into guilds the authors did not find significant differences for non-aphidophagous predators and parasitoids, whereas there were significantly and marginally significantly higher abundances of predators with aphids in their diet in isogenic maize stands in 2002 and 2003 respectively.

According to EFSA (2011d) data from other coleopteran-active Cry proteins do not indicate adverse effects on rove beetles, suggesting that they are not sensitive to the tested coleopteran-active Cry proteins. Porcar et al. (2010) reported that, based on a 15-day laboratory bioassay covering 70 % of the life-span of adults, the adult mortality of *A. coriaria* when fed a diet containing the coleopteran-active Cry3Aa protein, did not differ statistically from that of the control group. A field study in Hungary with Bt-maize that expresses the coleopteran-active Cry34/35Ab1 proteins showed that the overall assemblage of rove beetles was not significantly affected by the Cry34/35Ab1 proteins through their diet (Balog et al., 2011). These studies confirm that the adverse effects reported by Büchs et al. (2008) were likely due to varietal or prey quality effects, rather than the toxicity of the Cry3Bb1 protein.

Wold et al. (2001) did not find adverse effects of maize expressing Cry1Ab to *A. bipunctata* in laboratory and field studies, but reported some adverse effects on another coccinellid species in a laboratory test. Higher tier studies are also available in the literature and no adverse effects of Bt-maize expressing Cry1Ab on field populations of a range of coccinellid species were detected (e.g., Pilcher et al., 1997; Jasinski et al., 2003; Dively and Rose, 2004; Lundgren and Wiedenmann, 2005; Poza et al., 2005, Eckert et al., 2006; Alvarez-Alfageme et al., 2008, 2009, 2010). An important consideration in terms of environmental risk assessment is that it is unlikely that coccinellid larvae will be exposed to biologically relevant amounts of Cry1Ab from maize MON810. The exposure route used by Schmidt et al. (2009) may constitute a useful model for laboratory studies, but any exposure through egg feeding in the field is very unlikely. The Cry1Ab protein content in maize MON810 pollen (which is likely to be the most common source for possible toxin ingestion for coccinellids) is very low and ranges between 1-97 ηg/g fw (Nguyen and Jehle, 2007). Bt-proteins are normally absent in aphids feeding on maize (Head et al., 2001; Raps et al., 2001), which is the main diet of coccinellid larvae. However in contrast to maize, Burgio et al. (2010) report that a small fraction of Bt-protein could be found in aphids sucking on Bt oilseed rape. However, Romeis and Meissle (2010) argue that aphids do not ingest considerable amounts of insecticidal proteins when feeding on Bt-transgenic crops and that occasional reports of low concentrations of Bt Cry proteins in aphids can be explained by contamination of the samples.

Under field conditions Wold et al. (2001) found smaller numbers of ladybird beetle *Coleomegilla maculata* on Bt maize expressing Cry1Ab in only one of two years. The authors concluded: “In summary, few statistical differences and inconsistent numerical trends might suggest that Bt has no adverse effects on beneficial insects in the field”. Other studies regarding coleopteran species reported no effects either of lepidopteran specific Cry proteins (e.g. Lozzia, 1999, Bourguet et al., 2002, Candolfi et al., 2004, Toschki et al., 2007) or of coleopteran specific proteins (e.g. Lundgren and Wiedemann, 2002, Bhatti et al., 2005, Mullin et al., 2005, Lundgren et al., 2005, Ahmad et al., 2005, 2006a, Duan et al., 2006, 2008, Ferry et al., 2007, Raybould et al., 2007).

Cry1A and Cry3 effects have been reported from tier 1 or tier 2 studies on NTO species which are not related to the Lepidoptera or Coleoptera. These include aphids (Deml et al., 1999, Ashouri et al., 2001, Liu et al., 2005), acari (Obrist et al., 2006a), Heteroptera (Ponsard et al., 2002) and bees (Ramirez-Romero et al., 2005) (see also review of Hilbeck and Schmidt 2006). However none of these studies could clearly identify the Cry protein as factor responsible for the effect. The methodology and experimental design of some of the studies are subject to controversial discussions, e.g. those studies which reported adverse effects on predators and parasitoids fed with prey or hosts reared on Cry protein containing diet or plants (e.g. Hilbeck et al., 1998a,b, 1999, Lövei and Arpaia, 2005, Hilbeck and Schmidt, 2006). These studies are considered in more detail under Section 4.4.3 (tri-trophic interactions). No non-target effects of Cry proteins on bees were reported from a number of laboratory semi-field and field studies (Malone et al., 2001, Hanely, 2003, Malone, 2004, Bailey, et al., 2005, Babendreier et al., 2004, 2005, Rose et al., 2007, Marvier et al., 2007, Malone and Burgess, 2009), or for different groups of phytophagous and predatory arthropods (e.g. Riddick et al., 2000, Bourguet et al., 2002, Jasinski et al., 2003, Candolfi et al., 2004, Carter et al., 2004, Rauschen et al., 2004, Sisterson et al., 2004, 2007, Alvarez et al., 2005, Dively, 2005, Eckert, et al., 2006, Meissle and Lang, 2005, Naranjo et al., 2005a,b, Obrist et al., 2005, O’Callaghan et al., 2005, Pons et al., 2005, Poza et al., 2005, Eizaguirre et al.,2006, Habustova et al., 2006, Ludy and Lang 2006, Rodrigo-Simón et al., 2006, Habustova et al., 2007, Rose and Dively 2007, Toschki et al. 2007). In a field study where colonies foraged on Cry1Ab expressing maize (event Bt11) and were fed Bt-pollen cakes for 28 days, Rose et al. (2007) did not observe adverse effects on bee weight, foraging activity, and colony performance. Similarly, in a flight cage study maintained under controlled conditions, no significant differences were reported in honeybee mortality, syrup consumption and olfactory learning performance when honeybee colonies were exposed to different syrups containing Cry1Ab protoxin (Ramirez-Romero et al., 2005). In this respect, Ramirez-Romero et al. (2008) recently concluded that negative effects of the Cry1Ab protein on foraging behaviour and olfactory learning performance of honeybees are unlikely in natural conditions. Feeding behaviour and olfactory learning performance were disturbed only when honeybees were exposed to extremely high concentrations of Cry1Ab protein (5000 µg/kg), which do not occur under normal conditions (Ramirez-Romero et al., 2008). Lima et al. (2010) exposed honey bee larvae to either pure larval diet (control), diluted larval diet (diluted control) or larval diet diluted in a Cry1Ac solution at a concentration compatible with the maximum possible field exposure to Bt cotton. Although the diluted diet slightly increased larval mortality, Cry1Ac ingestion did not affect survival, developmental time, and neither adult body mass nor size, indicating that GM plants are unlikely to significantly impair the development of honey bee larvae. The larval-rearing system reported here was suitable to assess the lethal and sub-lethal effects of GM expressed toxins on honey bee larvae.

A different effect was observed when bees are exposed to a combination of Cry1Ac and CpTI (Cowpea Trypsin Inhibitor) in GM cotton (CCRI41) which is increasingly planted throughout China. Han et al. (2011) reported that Cry1Ac/CpTI pollen carried no lethal risk for honey bees. However, during a 7-day oral exposure to the various treatments (transgenic, imidacloprid-treated and control), honey bee feeding behaviour was disturbed and bees consumed significantly less CCRI41 cotton pollen than in the control group in which bees were exposed to conventional cotton pollen. It may indicate an antifeedant effect of CCRI41 pollen on honey bees and thus bees may be negatively affected if large areas are planted with such GM Bt cotton. After evaluating the recent studies by Álvares-Alfageme et al. (2010) and Porcar et al. (2010) and other known data from field trials, the German Biosafety Commission (ZKBS) came to the conclusion that the Bt proteins Cry1Ab and Cry3Bb1 are not expected to have potential adverse affects on ladybird beetles (ZKBS 2011). So far, only the publication by Schmidt et al. (2009), which is based on a laboratory study, has reported adverse effects on two-spotted ladybirds. This study was intensively criticized (Rauschen, 2010a, Ricroch et al., 2010). Hilbeck et al. (2012a,b) reiterated that their laboratory studies provide indications for possible hazards that require further investigation (or possibly long-term field monitoring) to determine whether they pose a risk or translate into ‘harm’ in the field. However, in the light of available data (e.g. reviewed by EFSA, 2009a, 2011c,d) and the low level of environmental exposure, the risk to ladybirds from Cry expressing Bt maize varieties is negligible. Moreover, Tian et al. (2012) demonstrated that Cry1F protein did not affect important fitness parameters of a ladybird beetle, *Coleomegilla maculata* experimentally exposed to high doses via a Cry1F resistant Lepidopteran pest.

In the EU, no negative impact of Cry3Bb1 expressing maize MON 88017 was shown on the abundance of spiders (Svobodová et al., 2012), carabids (Priesnitz, 2010; Svobodová et al., 2012), chrysomelids (Rauschen et al., 2010a), coccinellids (Rauschen et al., 2010a), staphylinids (Svobodová et al., 2012), and the hemipteran species *Trigonotylus caelestialium* (Rauschen et al., 2009) and *Zyginidia scutellaris* (Rauschen et al., 2008, 2010b). The results of these higher-tier studies confirm the conclusions of lower-tier studies, indicating that the Cry3Bb1 protein has little or no activity on species other than chrysomelids (EFSA, 2011d).

Similar results were optained from biosafety research Bt cotton in China. Chen et al. (2010) showed that the adult beetle abundance in the field did not differ significantly between non-GM and GM cotton, the exposure to Cry1Ac/CpTI pollen in laboratory had no effect on the developmental time, hatching rate, pupation rates and emergence rates of *H. luteolus*, no significant differences were found in the mating rates and the fecundity between treatments, and the survival curves of both treatments also resulted in no significant differences. It indicated there were no significant direct adverse effects of GM cotton pollen on *H. luteolus* both in the field and in the laboratory.

For Cry1F expressing GM maize, Virla et al. (2010) observed in field experiment that the population of the leafhoppers was higher in the GM maize than in the non-Bt control. Possible hypotheses for the differences in abundance are: a) that pleiotropic effects of Bt maize could attract adult leafhoppers; b) the existence of a possible direct competition between the leafhoppers and the target pest in order to utilize maize plants as refuge and feeding sites; and/or c) a differential attack of natural enemies occurring in non-Bt plots. However, no direct toxic effect on leafhoppers of Cry1F maize was observed.

Additionally, potential toxic effects of Cry proteins were also examined in studies on NT soil organisms. However, results of studies on the effects of Cry proteins on nematodes are controversial. When using Cry5B, Cry 6, Cry 12, Cry 14 and Cry 21 proteins direct toxic effects on nematodes were observed by Marroquin et al. (2000) and Wei et al. (2003), but these Cry proteins are different from the lepidopteran or coleopteran specific proteins currently used in GM plants in Europe. No significant differences in the abundance of nematodes in the rhizosphere of Bt maize and non-Bt maize have been reported (Saxena and Stotzky 2001a). In contrast, Manachini et al. (2004) reported a shift in nematode community structure in soil cultivated with Bt oilseed rape compared to the non-Bt oilseed rape control.

Griffiths et al. (2005) found a significantly reduced number of nematodes under Bt maize compared to non-Bt maize when they made an overall comparison of Bt versus non-Bt maize (MON810) across three different field sites in different European regions. But the authors judged this effect as small and within the normal variation range expected in the considered agricultural systems. In support of this conclusion, a greenhouse study observed no negative effects on nematodes and the nematode population sizes under Bt maize were higher than in soils with non-Bt maize (Griffiths et al., 2006).

The nematode *Caenorhabditis elegans* exposed to aqueous Cry3Bb1-containing solutions showed a dose-dependent inhibitory effect on the growth and reproduction, with EC50 values of 22.3 mg/L (0.29 µM) and 7.9 mg/L (0.10 µM), respectively, indicating susceptibility to the aqueous solution of the Cry3Bb1 protein (Höss et al., 2011). Higher-tier field studies conducted with Cry3Bb1-expressing maize in the EU (event MON 88017; Höss et al., 2011) and the USA (event MON 863; Al-Deeb et al., 2003) did not indicate significant differences in the abundance and diversity of nematodes in soil planted with Bt-maize and soil planted with its near-isogenic counterpart due to the low Cry3Bb1 protein concentrations in soil. Any effects on nematodes by Cry3Bb1-expressing maize and their products are likely to be minor compared with effects of agricultural practices, environmental stresses or differences between localities and maize varieties (e.g., Griffiths et al., 2005, 2006, 2007a,b).

According to Bakonyi et al. (2006) Bt-maize (MON810 producing Cry1Ab protein) was a less preferred food source for *Folsomia candida* (Collembola) than the isogenic control variety. No similar phenomenon was found in the studies with two other species (*Heteromurus nitidus* and *Sinella coeca*). Later, Bakonyi et al. (2011) reported the absence of adverse effects taken from long-term laboratory studies with several generations feeding of *F. candida* on MON810 maize. Bitzer et al. (2005) observed no detrimental effect on Collembola due to Bt maize cultivation. Heckmann et al. (2006) reported no adverse effect of Bt maize on the collembolan *Protoaphorura armata*. In general, no negative effects of Cry proteins on collembolans and soil mites have been reported in the scientific literature (reviewed by Icoz and Stotzky, 2008). The survival and reproduction of *Folsomia candida* fed leaf material of Cry3Bb1-expressing maize was not adversely affected by the Cry3Bb1 protein (EPA, 2010). The conclusion of the lower-tier studies was supported by higher-tier studies on Collembola performed with maize MON 88017 in the EU (Höneman et al., 2008), maize MON 863 in the USA (Al-Deeb et al., 2003; Ahmad et al., 2005; Bitzer et al., 2005), and Bt rice in China (Bai et al., 2011). No adverse effects of Cry3Bb1-expressing maize were reported on field densities of springtails and soil mites (Acari) in a nine months leaf litter-bag field study conducted in Switzerland with maize MON 88017, as compared with the non-Bt-treatment (Hönemann et al., 2008). Field trials conducted in the USA also showed that there were no significant differences in numbers of soil mites (Al-Deeb et al., 2003; Ahmad et al., 2005).

Zwahlen et al. (2003b) published results from a 200-day study investigating the impact of genetically modified Bt maize event Bt11 (expressing Cry1Ab) on immature and *adult Lumbricus terrestris* in a single worst-case laboratory study and in a single small scale field test. At the end of the laboratory test the earthworms showed a significant weight loss of 18% (compared with their initial weight) when fed with Bt maize litter whereas a weight gain of 4% occurred with non-GM control maize. No difference was found in a small scale field test. Two other studies reported that Cry1Ab had no apparent effect on earthworms or nematodes in a 45-day combined laboratory and field study (Saxena and Stotzky 2001a, Ahmad et al., 2006b). In addition, Vercesi et al. (2006) and Schrader et al. (2008) found no adverse effects on earthworms. Other laboratory studies were carried out to investigate effects of genetically modified Cry1Ab (MON810) maize leaf material on the terrestrial isopods, and a difference in isopod growth was reported (Escher et al., 2000, Wandeler et al., 2002, Clark et al., 2006). Another study reported potential sublethal effects of Cry-proteins on saprophytic diptera larvae (Büchs et al., 2005). No adverse effects due to toxicity of the Cry3Bb1 protein have been detected in lower-tier studies on the the enchytraeid worm species *Enchytraeus albidus*

(Hönemann and Nentwig, 2009). Feeding *E. albidus* with diets containing leaf material from maize MON 88017 did not affect the survival and reproduction of adults (Hönemann and Nentwig, 2009).

In their higher-tier field study conducted in the USA, Zeilinger et al. (2010) did not observe significant differences in numbers and biomass of juvenile and adult earthworms (*Aporrectodea caliginosa*, *A. trapezoides, A. tuberculata*, and *L. terrestris*) between non-Bt-maize and Bt-maize (event MON 863) varieties during four years of cultivation. A nine month leaf litter-bag field study conducted in Switzerland with maize MON 88017 revealed no difference in decomposer communities (including *Clitellata* which represented 6 % of the total abundance of observed decomposers) when compared with the near-isogenic counterpart and other conventional maize varieties (Hönemann et al., 2008).

The ECOGEN project found no effect of the Bt maize (MON810) on snails, microarthropods or mycorrhizal fungi in mesocosm and field experiments (Cortet et al., 2007, de Vaufleury et al., 2007, Griffith et al., 2007a, b, Krogh et al., 2007). One noticeable result was reported as the Cry protein was carried over into the soil food web by snails and their faeces (Harwood et al., 2005, Harwood and Obrycki, 2006, de Vaufleury et al., 2007). An extensive soil ecological field sampling programme was run throughout the ECOGEN project (Krogh and Griffiths, 2007) for soil microorganisms (Bacteria), microfauna (Protozoa and nematodes), mesofauna (Collembola, mites, enchytraeids) and macrofauna (earthworms). For all major groups of soil organisms no differences were observed with Bt-maize that were greater than differences caused by season, soil type, tillage practice or cultivar (Krogh and Griffiths, 2007).

The soil ecological evaluations in Krogh and Griffiths (2007) were performed at three levels of biological organisation: single species, mesocosm model communities and field ecosystems and produced specific conclusions at each level of complexity:

- Soil organisms held singly in lab cultures did not respond negatively to either pure Cry-toxin or maize plant material expressing Cry-toxin.
- Mesocosm experimental test systems responded mainly to properties of maize varieties other than the Cry-toxin and applied pesticides.
- Changes in soil biodiversity were detected mainly from tillage practices, soil types, crop type and history, pesticides and the maize variety.

Harwood and Obrycki (2006) studied the impact of Bt maize on molluscs e.g. *Deroceras reticulatum* which are readily consumed by many generalist predators so that predators would be exposed to higher Cry1Ab protein concentrations. The authors tested the hypothesis that slugs fed Bt maize would accumulate detectable quantities of Cry1Ab proteins for prolonged periods of time. It was possible to detect Cry1Ab proteins in slugs up to 95.9 h after consumption of Bt-maize. In addition Kramarz et al. (2007a,b) observed that Bt had an adverse effect on snails parasitized by nematodes whereas non-parasitized snails showed no reaction on Bt when healthy. However, these effects seem to have little or no ecological or biological relevance.

The Cry3Bb1 protein was detected in the gut and feaces of the molluscs, *Arion lusitanicus* and *Deroceras reticulatum*, after the slugs had fed on leaves from maize MON 88017, indicating possible exposure of slugs when feeding on Bt-maize. Following exposure, no differences in weight gain or loss of slugs were observed among the treatment groups (Zürbrugg and Nentwig, 2009). In a continuation of the study by Zürbrugg and Nentwig (2009) with experiments lasting for 16 weeks, no significant effects of maize MON 88017 were detected on the survival, weight change and oviposition of the slug *Arion vulgaris* (Hönemann and Nentwig, 2010).

Since the early 1990s, the potential harm that Bt insecticide posed to aquatic ecosystems has been studied (Kreutzweiser et al., 1992, Richardsen and Perrin, 1994 Kreutzweiser et al., 1994, Kreutzweiser and Capell, 1996). Overall, results from these studies indicated that significant adverse effects of Cry protein on aquatic macro-invertebrates are unlikely.

Two studies merit further attention: The study of Douville et al. (2007) demonstrated that aquatic organisms could also be exposed to Cry protein from genetically modified plants due to Bt crop residues in the water and sediments. A laboratory study by Rosi-Marshall et al. (2007) reported significant adverse effects on caddiesflies at high pollen exposure level, but the relationship to Bt maize events or dose-response level of Cry1Ab is unclear from the data presented in the publication. The EFSA GMO Panel considered that important background information on levels of exposure and plant material used is missing and that the conclusions made by Rosi-Marshall et al. (2007) are not supported by the data presented in the paper (EFSA, 2007c). Similar views were also expressed by ACRE (2007a), Beachy (2008) and Parrot (2008). Hence, it could be concluded that a potential hazard for Trichopterans has been identified under laboratory conditions when exposed to high doses of Cry proteins. However, due to the low level of Cry proteins in aquatic systems, as reported by Douville et al. (2005, 2007), exposure of Trichopterans in aquatic ecosystems is likely to be very low (Chambers et al., 2007). The publication by Douville et al. (2009) is a preliminary study on the occurrence of cry1Ab genes from Bt and Bt-maize in mussels without any indications of environmental impact. In addition, Bt proteins can have sublethal effects on non-target aquatic taxa, but this evidence should be considered in the context of the low levels of exposure and other anthropogenic impacts and alternative methods of pest control influencing streams draining agricultural regions (Chambers et al., 2010).

According to EFSA (2011d) few studies, assessing the impact of the Cry3Bb1 protein on non-target aquatic arthropods and the fate of the Cry3Bb1 protein in senescent and decaying maize detritus in aquatic environments, have been reported in the scientific literature so far, but data are available for the daphnid species *Daphnia magna* (APHIS, 2005; EPA, 2010), the dipteran species *Chironomus dilutus* (Prihoda and Coats, 2008a) and *Tipula abdominalis* (Jensen et al., 2010), the caddisflies *Lepidostoma* spp. and *Pycnopsyche scabripennis* (Jensen et al., 2010), and the isopod *Caecidotia communis* (Jensen et al., 2010). Based on exposure estimates, Carstens et al. (2011) identified shredders as the functional group most likely to be exposed to Cry proteins.

- No adverse toxic effects on *D. magna* were observed when fed high amounts of Cry3Bb1-expressing maize pollen mixed with water (APHIS, 2005; EPA, 2010). Questions have been raised about using maize pollen in aquatic invertebrate testing with *D. magna* because maize pollen is thought to be too large for ingestion by these filter feeders (EcoStrat, 2000; see also Bern, 1990) and, if ingested, to have a low food value for daphnids (Masclaux et al., 2011). However, there is some observational evidence that daphnids do ingest pollen (see Hadden, 1978 cited in Campbell, 1999). Daphnids fed maize pollen are actually yellow in colour, which can be indicative of ingestion of the test material, with no treatment mortality or behavioural change compared with untreated controls. Even though there is some observational evidence that daphnids do ingest pollen, there is no clear evidence that these filter feeders are capable of digesting pollen grains. The presence of a refractory wall reduces the digestibility of intact pollen grains by daphids, and hence the nutritional value of pollen for these filter feeders (Masclaux et al., 2011). Therefore, only a statement of no effect from exposure to pollen, and no statement on lack of toxicity can be made from the *D. magna* study provided by the applicant (EPA, 2010).

- Bøhn et al. (2008, 2010) revealed that *D. magna* fed a 100 % suspension of maize MON 810 flour under lower-tier conditions had a higher mortality and reduced fitness performance, as compared with the non-Bt-maize treatment, suggesting toxic effects of the Cry1Ab protein. However, it remains unclear whether the unusual delays in development of *D. magna* fed non-Bt-maize have been caused by nutritional deficiencies related to the maize-based diet or the presence of the Cry1Ab protein (EFSA, 2009d; Ricroch et al., 2010).

- Prihoda and Coats (2008a) observed a decrease in survival but no effect on the growth of the larvae of the *C. dilutus* when exposed to the Cry3Bb1 protein via root extracts of maize MON 863. However, the authors of the study concluded that it remains unclear if the observed effects were due to the presence of Cry3Bb1 or other compounds in the root extracts, as no control treatments with increasing concentrations of non-Bt maize root extracts were included.

- No adverse effects on non-target aquatic shredding arthropods (two caddisflies (*Lepidostoma spp.* and *P. scabripennis*), a crane fly (*T. abdominalis*) and an isopod (*C. communis*)) were reported when fed senesced leaf tissues from Cry3Bb1-expressing maize (maize event MON 810 x MON 863) ad libitum for 30 days (Jensen et al., 2010; Lamp, 2010).

- Direct feeding studies with MON810 maize expressing Cry1Ab proteins did not show consistent effects on salmon and zebrafish (Sissener et al., 2010).

In summary, exposure of non-target organisms to Cry proteins in aquatic ecosystems is likely to be very low (Douville et al., 2005, 2007; Wolt and Peterson, 2010; Carstens et al., 2011) and hazardous effects are unlikely.

New Bt crop developments concern Vip3A, which is a Bt vegetative insecticidal protein that is active against lepidopterous pests (Raybould and Vlachos, 2010). Vip3A has a different mode of action from other proteins for control of Lepidoptera, and when combined with these proteins in new Bt crops, it is expected to delay the evolution of pest resistance to Bt crops. Raybould and Vlachos (2010) presented data on the effects of Vip3A on non-target organisms, and an ecological risk assessment of MIR162 maize, which expresses Vip3Aa20. Their laboratory studies indicated few adverse effects of Vip3A to non-target organisms: 11 of 12 species tested showed no adverse effects when exposed to high concentrations of Vip3A relative to estimated exposures resulting from cultivation of MIR162 maize. *Daphnia magna* exposed to Vip3Aa20 were unaffected in terms of survival or fecundity, but grew slightly more slowly than unexposed controls. The data of Raybould and Vlachos (2010) indicate that cultivation of MIR162 maize poses negligible risk to non-target organisms, and that crops producing Vip3A are unlikely to adversely affect biological control organisms.

GM crops expressing toxic proteins such as protease inhibitors, chitinase or lectins have been reported as causing adverse effects on bees, predators and parasitoids (e.g. Malone et al., 1999, Burgess et al., 2002, Down et al., 2003, Otsu et al., 2003, Dechaume-Moncharmont et al., 2005, Lövei and Arpaia 2005, Romeis et al., 2006b, Malone and Burgess 2009). However none of these GM crops has been commercialised yet.

Von Burg et al. (2010) studied the impact of GM wheat lines on clonal aphids within the NFP59 research project. Their results showed that the genetically modified plants used were of similar host plant quality as the non-transformed control lines and that the introduced transgene had no major effect on the performance of individual aphid clones. Another test system for examining effects of GM mediated fungal resistance was used for the GM wheat. Lindfeldt et al. (2011) studied enchytraeids as an important model decomposer species in feeding experiments with transgenic and non-transgenic GM wheat diets, to check for possible effects on survival as well as reproduction of *Enchytraeus albidus*. Results indicated that effects due to specific resistance were either absent or too weak to affect number of surviving adults or numbers of offspring in a significant way. Chitinase and glucanase expression affected the number of surviving adults negatively, but a comparison of five conventional wheat varieties also revealed significant differences in the number of surviving adults. Since these differences were in the same range as the effect of glucanase and chitinase expression, Lindfeld et al. (2011) questioned whether the negative transgenic effect is of ecological relevance or whether other biotic, abiotic and genetic factors are more important. The analysis of plant compound composition showed no differences between transgenic and non-transgenic wheat varieties: No correlation to number of surviving adults or number of offspring was observed for lignin, hemicellulose, and cellulose contents. Peter et al. (2010) found occasional differences in development time or the number of offspring for two dipteran species in several investigated GM wheat diet comparisons but found no consistent pattern of GM wheat effects on the tested dipteran species. Though some wheat lines showed an altered chemical composition, possibly indicating a pleiotropic effect, they found no correlation between diet quality of the wheat lines and fitness parameters of the two Diptera larvae. Direct adverse effects on NTOs caused by herbicide tolerant events in GM crops have not been reported (e.g. Volkmar et al., 2003, Huang et al., 2004, EFSA, 2011d). However the herbicides and their management can affect NTO populations as described in Section 4.7.

In future, GM crops expressing multiple or altered Cry-proteins will be widely used, for example Bt cotton expressing Cry1Ac and Cry2Aa proteins both acting against lepidopteran pests (Jurat-Fuentes et al., 2003), maize MON89034 expressing the Cry1.105 protein, and maize 59122 expressing Cry34 and Cry35. Other approaches may use other classes of pest defence mechanisms (e.g. barley cystatins to interfere with the performance of two aphid species Carillo et al., 2010). So far there is no indication that there are changes in specificity of cry toxins or synergistic effects on NTOs from combinations of Cry proteins.

Conclusions regarding direct effects on NTOs:

- An extensive body of research data is available for effects of GM plants on non-target organism.
- Where the available literature indicates adverse effects on non-target of crops expressing Cry toxinsin Tier 1 and Tier 2 studies, these effects are rarely found in Tier 3 and Tier 4 studies, i.e. toxic effects observed in the laboratory do not necessarily translate to effects on populations under field conditions, in most cases due to low levels of exposure.
- However, conclusions from short-term ecotoxicological experiments cannot entirely eliminate uncertainty regarding long-term environmental effects. In particular, the observed sublethal effects could have the intrinsic potential to affect NTOs in the long run.
- The majority of laboratory studies and all the field studies reviewed in the BEETLE (2009), Carpenter (2011), and EFSA (2011c,d) reports and by the authors of this study demonstrate that adverse or long-lasting effect on NTOs are likely to be rare.
- In some instances (e.g. high intensity of cultivation of Bt maize) exposure to pollen from Bt maize may adversely affect populations of non-target Lepidoptera that are extremely sensitive to Cry1 toxins.
- There are no indications of direct adverse effects on NTOs caused by HT events, however removal of weeds can lead to food chain effects as discussed in Section 4.6.

4.5.2 Effects on NTOs due to Altered Nutritional Composition of the GM Plant

There is ongoing discussion in the literature whether genetic modification leads to unanticipated or unintended effects on the nutritional composition of GM plants which might affect NTOs. Unintended changes are defined as consistent differences between the GM plant and its appropriate comparator, which go beyond the primary intended changes of introducing the transgene(s) (EFSA, 2010a,b). For example, Saxena and Stotzky (2001b) reported higher lignin contents in three GM maize events (maize Bt11, Bt176, and MON810). In addition, Poerschmann et al. (2005) confirmed the occurrence of altered lignin biosynthesis in stems of Bt maize as described by Saxena and Stotzky (2001b), but the differences between the GM plants and their isogenic controls seemed to be much less than originally reorted by Saxena and Stotzky (2001b). In contrast, Jung and Sheaffer (2004) suggested that the extent of lignification of Bt maize (several lines derived from MON810 and Bt11) does not differ from the non-GM controls. Since lignin is well-known for its capability to influence palatability and digestibility of plant material to herbivores and decomposers (Zwahlen et al., 2003a, 2007), the possibility of altered lignin content in Bt maize needs to be compared with the available literature on whether adverse effects have been reported for maize herbivores or decomposers. It is not clear whether the effects of higher lignin in Bt maize are caused by the genetic modification, by potential epigenetic effects or by different genetic backgrounds of the GM crop and its comparators. However it is generally considered that alterned lignin content in maize varieties is not an effect attributed to the insertion of the transgene, but from the genetic background of the maize varieties under consideration (Fernie et al., 2006; Griffiths et al., 2007; Lehman et al., 2008, 2010; Zurbrügg et al., 2010; Yanni et al., 2011). In the ECOGEN project, Bt-maize (MON810) had no differences in biological decomposition in any of the three locations investigated over several years in Denmark and France concerning the decomposition of organic matter as studied using a straw-litterbag methodology (Krogh and Griffiths, 2007). These results are confirmed be other studies (Lehman et al., 2008).

Interestingly, some studies reported that herbivores might even prefer genetically modified plants as food more than their conventional counterparts or developed faster on GM crops than on non-GM crops. This effect – contrasting the previous reports on higher lignin content of Bt maize – was observed in lab studies for acari and isopods (Escher et al., 2000, Wandeler et al., 2002, Zemková-Rovenská et al., 2005) and in field studies for aphids, thrips and saprophytic beetles (Bourguet et al., 2002, Lumbierres et al., 2004, Eckert et al., 2006). There are also contrary data by Akhtar et al. (2010) who showed that Bt rice lines may be less preferable host plants for thrips in comparison to the non-Bt rice plants, but it is unclear what the reason for this effect is.

Starch modified potatoes developed by BASF were also studied to determine whether plant-associated organisms (e.g. invertebrates) might be affected by altered nutritional composition of the host crop (EFSA, 2006). Data are available on the impact of the modified crops on plant-associated organisms from field studies carried out in Sweden, Germany and The Netherlands, suggesting that environmental impacts are small or absent. According to EFSA (2006) the results of field studies indicate no change in susceptibility to pests (e.g. aphids, leafhoppers, potato cyst nematodes (sp *Globodera*)) and diseases (e.g. late blight (*Phytophthora infestans*), potato early blight (*Alternaria solani*), *Erwinia* rots) than non-GM potato lines. There was no evidence of changes in sensitivity to the plant-associated viruses PVY, PLRV, PMTV, and TRV. In view of this and the equivalent composition of the GM potato plant, EFSA (2006) considered that no adverse effects on plant-associated organisms would be expected from cultivation of the potato EH92-527-1.

Trees are currently being modified to produce higher or lower lignin contents. Potential adverse effects of such GM trees were studied by Halpin et al. (2007) and Tiimonen et al. (2005, 2007) and no unexpected biological or ecological impacts were detected. Interactions with leaf-feeding insects, microbial pathogens and soil organisms were unaltered although the short-term decomposition of genetically modified roots was slightly enhanced (Halpin et al., 2007).

In applications for the release of Bt and HT maize no altered agronomic and phenotypic characteristics, except for the specific target pest resistance and herbicide tolerance have been identified, suggesting that unintended effects are rare in the GM maize screened by plant breeders. Based on data on molecular characterization, compositional analysis, field performance and data on plant-environment interactions it is estimated that unintended environmental effects of GM plants will occur at a similar or lower frequency than in conventionally bred plant varieties.

Conclusions regarding effects on NTOs due to altered nutritional composition:

- It can be anticipated on a case by case basis that the nutritional value of GM crops for herbivores may potentially be altered by the genetic modification.
- There is currently no indication that altered starch composition changes populations of plant associated herbivores or decomposers e.g. due to the genetic modification (amylose or amylopectin content) of potato tubers.
- There are no indications to date that GM crops with changed composition alter herbivore attractiveness and no adverse effects have been reported.

4.5.3 Tritrophic Interactions on NTOs

The ERA of NTOs is also concerned with tri-trophic interactions since first evidence was found that predatory green lacewing *Chrysoperla carnea* may be adversely affected by feeding on herbivorous larvae feeding on Bt maize (Hilbeck et al., 1998a,b, 1999, and recently reviewed by Hilbeck and Schmidt (2006). These observed indirect effects from prey feeding on Bt maize or Bt protein have been widely discussed and Romeis et al. (2004), Dutton et al. (2003, 2005), Rodrigo-Simón et al. (2006), and Li et al. (2008) concluded that the observed mortality in Bt-fed lacewing larvae was solely due to lower nutritional quality of the sublethally affected prey and not an affect of the Cry protein derived from the Bt plant. Based on an additional literature review, Romeis et al. (2006a) emphasized that laboratory and glasshouse studies have revealed effects on predators only when Bt-susceptible, sublethally harmed herbivores of bad nutritional quality were used as prey or host, with no indication of direct toxic effects. Conversely, Hilbeck and Schmidt (2006) considered this unlikely and a too limited interpretation as they also demonstrated adverse effects of the Bt protein fed directly to the predator using a specific lacewing diet. Lately, Lawo et al. (2010) produced clear evidence that nutritional prey-quality factors rather than the Bt protein, underlie the observed negative effects when *C. carnea* larvae are fed with Bt cotton-fed prey. Possible factors were an altered sugar composition or fitness costs associated with the excess intake of other nutrients. It is thus not surprising that most regulatory risk assessors consider that tri-trophic interactions thus far shown are more likely to be affects due to changes in prey availability or quality. The use of appropriate methodology is essential for generating meaningful results from tri-trophic experimental studies.

The potential carry over of Bt proteins in the food chain has also been considered by several other studies of predator species. Carabid beetles, Heteroptera, and Chrysopidae were exposed to surprisingly high concentrations of Cry1Ab Bt protein in spider mites (*Tetranychus urticae*) (Harwood et al., 2005, Obrist et al., 2006a, Zwahlen and Andow, 2005) which was three times higher than in the Bt176 maize leaves that the mites had fed on (Obrist et al., 2006b). No adverse effects were identified. It can be concluded that the main predator species are not susceptible to the Cry1 proteins currently used in a large variety of

GM crops. In addition, Li and Romeis (2010) conducted studies to assess the prey-mediated effects of Cry3Bb1-expressing Bt maize (event MON88017) on the ladybird beetle *Stethorus punctillum* (Coleoptera: Coccinellidae). The results indicate that *S. punctillum* is not harmed by feeding on spider mites containing Cry3Bb1. Consequently, detrimental effects on this predator when preying in Cry3Bb1-expressing Bt maize fields are unlikely.

Host quality can affect tritophic interactions and it has been shown that in some cases parasitoids or hyperparasitoids developed on Bt fed hosts were significantly smaller, had longer development times or less fecundity or were less successful in parasitizing (Romeis et al., 2003, Prütz et al., 2004, Lövei and Arpaia, 2005, Prütz and Dettner, 2004, Romeis et al., 2006b, Sanders et al., 2007, Ramirez-Romero et al., 2007).

In contrast, Liu et al. (2011) did not find an effect on the parasitoid, *Diadegma insulare*, using a system of Cry1Ac expressing Bt broccoli plants with susceptible or Cry1Ac-resistant *Plutella xylostella.* Lumbierres et al. (2010) studied the effect of Bt maize on aphid parasitism and the aphid–parasitoid complex in field conditions on three transgenic varieties, two derived from event MON810 and one from Bt176, and their near-isogenic lines in a two-year study. No differences in aphid abundance were found between Bt maize varieties and their near-isogenics. Bt maize did not alter the aphid-parasitoid associations and had no effect on the aphid parasitism and hyperparasitism rates. The results suggest that Bt maize has no negative impact on second, third and fourth levels of the trophic relationships studied.

Birch et al. (1999) showed that a predatory 2-spotted ladybird, feeding on aphids reared on potatoes expressing snowdrop lectin, had reduced longevity and fecundity. In contrast, Down et al. (2003) found that the same prey and host species showed no effects on longevity and a trend for improved fecundity of up to 70%. Furthermore Schuler et al. (2003) reported that the number of emerging parasitoids was higher on Bt-plants compared to wildtype plants. Faria et al. (2007) reported in laboratory studies an effect of Bt maize on the performance of the maize leaf aphid *Rhopalosiphum maidis*, which in turn enhanced the performance of parasitic wasps that feed on aphid honeydew from GM plants.

Li et al. (2011) conducted a tritrophic bioassay to evaluate the potential impact of Cry2Ab- and Cry1Ac-expressing cotton on fitness parameters of the ladybird beetle, *Coleomegilla maculate,* using Bt-susceptible and -resistant larvae of *Trichoplusia ni* as prey. The ladybird larvae survival, development time, adult weight and fecundity were not different when they were fed with resistant *T. ni* larvae reared on either Bt or control cotton. Overall the results show that *C. maculata* is not affected by Bt cotton and is not sensitive to Cry2Ab and Cry1Ac at concentrations exceeding the levels in Bt cotton, thus demonstrating that Bt cotton will pose a negligible risk to *C. maculata*.

A tri-trophic study was performed with GM oilseed rape expressing a protease inhibitor (oryzacystatin – OC-1), the grey field pest mollusc *Deroceras reticulatum* as herbivore and *Pterostichus melanarius* as predator (Harwood and Obrycki, 2006). It was demonstrated that the protease inhibitor had no detrimental effect on the beneficial beetle consuming the pest mollusc, which was exposed to OC-1.

Trophic interactions between GMP and birds were studied by Gibbons et al. (2006) and by Chamberlain et al. (2007) based on the farm scale evaluation (FSE) in the UK (see Firbank et al., 2003a, 2006). The study aimed to compare bird abundance between GMHT and conventional crop treatments. The management of the GMHT plant and not the genetic modification itself showed effects on decrease or increase of bird abundance on a case-by-case and species-by-species basis. The observed differences were dependent on food availability (see also indirect effects of HT crops: section 4.7).

Many studies have looked at GM plant effects on single non-target herbivore species or on simple herbivore-natural enemy food chains (von Burg et al., 2011). Agro-ecosystems, however, are characterized by numerous insect species which are involved in complex interactions, forming food webs. In the NFP59 study by von Burg et al. (2011), GM disease-resistant wheat (*Triticum aestivum*) was studied in semi-field studies for its effect on aphid-parasitoid food webs. Allthough significant effects of the different wheat lines on insect community structure up to the fourth trophic level were observed, the effects were inconsistent between study years and the variation between wheat varieties was as big as between GM plants and their controls. These results suggest that the impact of powdery mildew-resistant GM wheat plants on food web structure may be negligible and potential ecological effects on non-target insects are expected to be limited.

Insect herbivores may change their host plant preferences as an ‘avoidance strategy’ to escape Bt plants, and this could result in predators also switching to prey on other plants. For example, an altered preference of prey was described by Zemková-Rovenská et al. (2005) showing that predatory mites preferred feeding on mites not reared on genetically modified Bt egg plants. Since the objective of GM IR plants is to reduce the abundance of the target (pest) organisms, decreased abundance of monophagous or oligophagous predators or parasitoids of these target pests can be expected (Riddick et al., 1998, Bourguet et al., 2002, Pilcher et al., 2005).

A meta-analysis of published field studies on non-target effects of Bt-crops (Wolfenbarger et al., 2008) differentiated the effects on functional guilds of non-target arthropods. The abundance of predators, parasitoids, omnivores, detritivores and herbivores was compared under scenarios where neither, only the non-Bt-crops, or both Bt and non-Bt-crops received insecticide treatments. Different effects of Bt-maize on functional guilds of non-target arthropods were reported. As expected, fewer specialist parasitoids of the target pest occurred in Bt-maize fields (specifically *Macrocentrus grandii*), as compared to unsprayed non-Bt-controls, but no significant reduction was detected for other parasitoids. Higher numbers of the generalist predator *C. maculata* were found in Bt-maize compared to non-Bt-maize, with no difference found for other common predatory genera.

Conclusions regarding tritrophic interactions on NTOs:

- The vast majority of available studies on predators or parasitoids with hosts feeding on GM plants, have reported effects within the normal variation and no adverse effects. In comparison chemical insecticide treatments were demonstrated to have greater adverse impacts on tritrophic level species (Marvier et al., 2007, Naranjo 2009).

- GM protein (e.g. “Bt-”) susceptible herbivores (2nd trophic level organisms) feeding on host plants expressing GM proteins (1st trophic level organism) could have reduced nutritional value for predators or parasitoids and potentially affect local populations of these 3rd or 4th trophic level organisms.

- Long-term adverse effects on populations or diversity of predators or parasitoids will also depend on a range of ecological factors, such as availability of alternative prey or area of GM plant cultivation, so that any affects are likely to be localized and temporary.

4.5.4 Effects on NTOs due to Persistence and Accumulation of New GM Compounds

Some scientific publications indicate that Cry proteins may persist at low concentrations in soil following cultivation of Bt maize. Cry protein persistence was hypothesed to negatively affect soil organisms, and there is also a debate whether the processes governing the fate and bioavailability of Cry proteins in soils are sufficiently understood (Madlinger et al., 2011, EFSA, 2011d,e). Therefore, risk assessment needs to consider both direct and indirect impacts of the protein or the Bt maize (e.g. potential increase of lignin content in combination with a possible delay in decomposition) on non-target soil organisms and soil functions (e.g. Saxena et al., 2002a, Zwahlen et al., 2003a).

There have been several studies of Cry protein persistence (e.g. Dubelman et al., 2005) including a large research project founded by the German Ministry of Science and Education (BMBF) in the years 2001-2004. In this project, Baumgarte and Tebbe (2005) and Nguyen and Jehle (2007) measured the tissue-specific expression and seasonal abundance of Cry1Ab protein in Mon810 maize plants and potential accumulation of the Cry protein in German soils. The authors specifically considered methodological constraints e.g. the re-extraction capability of Cry proteins from soil (Baumgarte and Tebbe 2005). The Cry1Ab protein did not accumulate during the growing season in the rhizosphere. The concentrations of the Cry1Ab protein in soil from Bt-maize fields were in the range between 0.1 and 10 ng g-1 in bulk soils and rhizospheres. Baumgarte and Tebbe (2005) were not aware of any non-target or target organism that would directly respond to such low concentrations as a bioindicator. In addition, the bacterial community structure was less affected by the Cry1Ab protein than by other environmental factors, i.e. the age of the plants or field heterogeneities. Gruber et al. (2011a) investigated the fate of Cry1Ab in soil under long-term Bt-maize cultivation in an experimental field trial performed over nine growing seasons on four South German field sites cultivated with MON810 and its near isogenic non Bt-maize variety. The Cry1Ab protein was never detected in soil sampled in the spring before the next farming season at any of the four experimental sites. There was no evidence for accumulation or persistence of Cry1Ab protein in different soils under long-term Bt-maize cultivation.

The EU funded project (ECOGEN) conducted over four years between 2002-2006 with field plots in Denmark and France (Andersen et al., 2007, Krogh and Griffiths, 2007) studied Cry1Ab protein concentrations in the soil cultivated with Bt maize and controls. Levels were increased in the plots with Bt-varieties but they did not seem to increase from year to year. The quantification of Cry-protein in soil was confounded by the low concentrations in soil and interference from soil factors, as suggested by the seasonal variation in the amounts of Cry-protein apparently detected even under non-Bt maize. In particular when comparing a range of varieties of Bt-maize and near-isogenic maize it was demonstrated that there were no detectable differences in the concentration of Cry-toxin in plant or soil with any of the Cry-expressing varieties. Although soil nematodes and microbial community structure differed between maize varieties, these could not be related to the Bt-trait (Krogh and Griffiths, 2007).

There is no evidence for accumulation of the Cry proteins on agricultural fields cultivated repeatedly with Bt maize (e.g. EFSA, 2011d), despite the protein's potential to bind to surface-active particles. Effects of crops on soil microbial communities, which are especially expected in the rhizosphere or on decaying plant material, depend more on their species, variety or age than whether they are genetically modified. Tan et al. (2010) showed that neither the actively growing Bt maize (MON810 expressing Cry1Ab and a Chinese variety expressing Cry1A) nor its straw had any constant apparent effect on soil bacteria and fungi community structure. According to the authors, the age of the growing plants, or the timing of plant straw decomposition may have more effect on the microbial community than other factors, i.e., the presence of Cry protein, plant hybrid and variety.

Rearrangements in structural diversity and population abundance of non-target soil organisms occur frequently in the agricultural environment. They are typically associated with several sources of variation, caused by natural variability (e.g. soil heterogeneity, weather conditions) and agricultural practices (e.g., soil tillage, crop rotation, irrigation measures) and are thus not necessarily an indication of environmental harm.

Another potential source of Bt protein entrance in soil ecosystems is via manure from animal feed Bt maize. Gruber et al. (2011b) examined the fate of recombinant Cry1Ab protein in a liquid manure field trial when feeding GM maize MON810 to dairy cows. A rapid decline of Cry1Ab levels was observed as 2.6% and 0.9% of Cry1Ab from the GM plant were detected in feed and liquid manure, respectively. Half of this residual Cry1Ab persisted during slurry storage for 25 weeks. After application to experimental fields, final degradation of Cry1Ab to below detectable levels in soil was reported. Cry1Ab exhibited a higher rate of degradation compared to total protein in the agricultural processes. The soil exposure via manure is therefore regarded as biologically irrelevant.

In Canada, Douville et al. (2007) examined the occurrence and persistence of Cry1Ab protein and the *Cry1Ab* gene from conventional Bt spray (Btk) and genetically modified Bt maize in aquatic environments near fields where Bt maize was cultivated in Canada. The Cry1Ab gene from Bt maize was still detectable after 40 days in clay and sand-rich sediments and both the DNA from the GM maize and from naturally occurring Bt was more abundant in the sediment than in the surface water. Cry1Ab DNA sequences were detected as far away as 82 km downstream from a maize cultivation plot, suggesting that there were multiple sources of this gene and/or that it undergoes transport by the water column. Tank et al. (2010) found that maize detritus is common in lowgradient stream channels in northwestern Indiana, USA. There, Cry1Ab proteins persist in maize leaves and can be measured in the water column even 6 month after harvest. Allthough Tank et al. (2010) were unable to conclude on the ecological consequences for stream-dwelling organisms that are exposed to the dissolved Cry1Ab concentrations, Wolt and Peterson (2010) used a conservative screening level approach to to evaluate the potential risk to sensitive aquatic species occurring in a representative agroecosystem. It was concluded the risk expressed in terms of the combined probability of short-term exposure and acute effects to a sensitive species indicated no concern in 99% of cases. Tank et al.,(2010) reported a mean Cry1Ab concentration (±SD) in stream water samples 14 ± 5 ng/L, with a maximum concentration of 32 ng/L. Wolt and Peterson (2010) used for their risk expression that the combined probability of exposure and effect occurring at or below 7200 ng/L would be manifested in 1% of cases.

There is no tendency for DNA accumulation as sediment-associated Cry1Ab gene from Bt maize decreased with distance from the Bt maize field. The data indicate that DNA from Bt maize and conventional Btk are persistent in aquatic environments and were detected in rivers draining farming areas. However, the levels of Cry1Ab protein in samples were mostly below the detection limit, and thus there is no indication for biologically relevant effects caused of the Bt maize plants.

In many countries GM Bt-maize will be used for agricultural biogas facilities. Rauschen and Schuphan (2006) monitored the fate of Cry1Ab proteins in silage and biogas production chains of two farm-scale biogas facilities in Germany. The Cry1Ab content in silage exhibited no clear-cut pattern of decrease over the experimental time of 4 months. After fermentation in the biogas plants, the Cry1Ab content declined to trace amounts of around 3.5 ng g^{-1} in the effluents. The limit of detection of the employed ELISA test corresponded to 0.75 ng Cry1Ab g^{-1} sample material. Assays with larvae of *O. nubilalis* showed no bioactivity of the reactor effluents. The authors conclude that the ecotoxicology is negligible considering the utilization of residual material for fertilizer in agriculture.

A four year study in Canada on the decay of genetically modified maize Bt protein was published by Hopkins and Gregorich (2003). The Bt protein decay followed the rate at which the Bt-maize leaves decomposed in soil from a field in which Bt-maize had been cultivated for four years. In addition, Hopkins and Gregorich (2005) determined the concentrations of the Cry1Ab protein in organic residues from MON 810 maize plants at increasing stages of ageing and decay, and the subsequent decomposition in soil of these residues and the Cry1Ab protein in them. The Cry1Ab protein decomposes faster than the bulk organic carbon in residues and it is likely to fall below the detection limit by ELISA within few months of entering the soil. The results suggested that much of the Cry1Ab protein in crop residues is highly labile and quickly decomposes in soil, but that a small fraction may be protected from decay in relatively recalcitrant residues.

In the USA, Icoz and Stotzky (2007) studied the potential accumulation of a Cry3Bb1 in sterile hydroponic culture and in nonsterile soil throughout plant growth (of GM Maize MON863). Soils were analyzed for the presence of the protein every 7 to 10 days with a western blot assay (ImmunoStrip) and verified by ELISA. The protein was detected for only a maximum of 21 days. These results again indicate that Bt proteins do not persist or accumulate in soil and seem to be degraded rapidly.

Icoz and Stotzky (2008) reviewed various reports on the persistence of Cry-proteins in soils. Half-lives of Cry1Ab ranged from 1.6 days (Sims and Holden, 1996) up to 34 days (Wang et al., 2006a). Long-term persistence of Cry1Ab is also variable: Low concentrations were detected ranging from 56 days (Donegan et al., 1995) to 234 days (Tapp and Stotzky, 1998) and 180 to 350 days in residues of Bt maize (Saxena and Stotzky, 2002). Recent studies confirm these observations (Marchetti et al., 2007).

As mentioned in chapter 4.4.2 Bt protein might accumulate in prey organisms which could lead to increased exposure of predators or parasitoids (Obrist et al., 2006a,b), but no direct toxic effects have been confirmed to date.

Conclusions regarding persistence and accumulation:

- There is no indication that Cry proteins are likely to accumulate in soil where Bt plants are cultivated even over several consecutive years.
- The time-spans over which low residue concentrations of Cry proteins have been detected vary and so the persistence and fate of Cry proteins in the soil is not fully understood.
- However, Cry-protein concentrations measured by standard ELISA-tests in soil or water are generally very low indicating that direct toxic effects to soil or water organisms are unlikely.

4.5.5 Effects on Rhizosphere Microbiota

The impact of GM plants on rhizosphere microbiota is an important consideration in biosafety assessments (Schuler, 2006). For example, saprophytic fungi and bacteria are key to the functioning of soil ecosystems, and exhibit a range of interactions with plants. In the case of GM plants producing Cry proteins, there are two pathways of exposure of rhizosphere microbiota: (i) decaying plant material (Baumgarte and Tebbe, 2005) and (ii) release of Cry1Ab proteins by roots via root exudates (Saxena et al., 2002b).

Hannula et al. (2011) found no detectable differences between a GM potato cultivar and its parental cultivar in terms of influence on fungal community structure of function. Fungal community structure and functioning of both GM- and parental cultivars fell within the range of other cultivars at most sampling moments.

Widmer (2007) analysed and reviewed 60 studies for the potential effects of seven different types of genetically engineered traits: herbicide tolerance, insect resistance, virus resistance, proteinase inhibitors, antimicrobial activity, environmental application, and biomolecule production. Widmer (2007) came to the conclusion that tools for the sensitive detection of changes in soil microbiological characteristics are available; however, they also reveal that at present it is very difficult or impossible to define which alterations in these characteristics may represent unacceptable damage to a soil system. A number of studies were published under the umbrella of the ECOGEN EU research project (for an overview see Krogh and Griffiths, 2007). The international consortium of French, UK and Danish scientists examined primarily MON810 Maize expressing the Cry1Ab insecticidal protein from *Bacillus thuringiensis*, and later GM herbicide tolerant (HT) maize was also included in some later stages aof the project. Mesocosm studies showed that small effects of Bt maize or conventional insecticide treatments on nematodes, protozoa and microorganisms were less pronounced than effects due to soil and plant growth stage (Griffiths et al., 2006), and less than the variation seen between eight maize cultivars (Griffiths et al., 2007b). No GM maize effects were observed on snails, microarthropods or mycorrhizal fungi. However, the Cry1Ab protein was detectable in snail faeces, and was therfore considered as a novel route of Bt protein exposure to soil food webs (de Vaufleury et al., 2007). Within ECOGEN, field experiments were also carried out at four sites across three European climatic zones and results of these field experiments indicated that MON810 maize could have a significant, but small and transient negative effects on soil protozoa, nematodes and microorganisms (Griffiths et al., 2005; 2007a). There was no indication for effects on organic matter (wheat straw) decomposition (Cortet et al., 2006).

The advantage of such an interdisciplinary project like ECOGEN is that experiments are conducted using the same organisms and soils across a range of scales (i.e. laboratory, glasshouse and field), which allows for comparison of scales and assessment of their utility for biosafety assessments. It was not possible to predict the outcome between scales, but there was useful information and insights from each of the experimental approaches (Birch et al., 2007). The complexity of soil organisms and their functioning was transformed into soil quality attributes and fed into a multi-attribute model, which now can be used in assessment of new agricultural technologies including GM crops. Details of this quantitative, multi-attribute model were published by Bohanec et al. (2007). The model considers the effects of different cropping systems on soil quality, and has considerable potential for application for other aspects of soil management.

The ECOGEN reseach group concluded that Bt-maize does not have deleterious effects on the soil biota. Although soil nematodes and microbial community structure differed between maize varieties, these could not be related to the Bt-trait (Krogh and Griffiths, 2007). The few ‘adverse’ effects observed were considered most likely to be caused by differences between the maize varieties. It is not the Bt-maize that influences soil quality, but the agricultural techniques used in association with the GM crop which could potentially improve (reduced tillage) or reduce (increased use of pesticides) the soil quality.

In addition Lilley et al. (2006) analysed various published studies on the effects of GM plants on soil systems: 25 peer reviewed studies were evaluated involving nine plant species (alfalfa, bird’s-foot trefoil, black nightshade, potato, rice, maize, cotton, tobacco and oilseed rape) genetically modified in ten distinct ways for the expression of: α-amylase; lignin peroxidase; an organic acid (malate dehydrogenase); T4-lysozyme (anti-pathogenic); cecropin b (an antibacterial lytic peptide); Bt protein; insect resistance (proteinase inhibitor I); herbicide tolerance; opines; and lignin production. The majority of the analysed studies were performed during a single growth-season, and some post-experimental monitoring was carried out. Two- and three-year studies were perfomed for GM herbicide tolerant canola and T4-lysozyme expressing potato. Lilley et al. (2006) noticed in 16 of 25 studies effects on the soil community or soil system. These effects were considered as being transient and of little biological consequence. Discribed effects were on (i) bacterial diversity, number and activity; (ii) fungal counts; (iii) effects on numbers of protozoa, nematodes and collembola; (iv) diversity of nematodes; and (v) woodlice mortality. Lilley et al. (2006) concluded:

(a) Considerations of the transgene product, its activity, site of expression and persistence are important guides to developing, on a case-by-case basis, lines of investigation into probable and/or important potential effects.

(b) Most GM plants have detectable effects on the soil system, but they are relatively minor compared with differences between cultivars or those associated with weather and season. Assays of the natural variation in the system are a valuable baseline reference.

(c) The response of soil systems when genetically modified plants are removed provides an important measure of impact. Studies generally find a quick return of many soil parameters to match those of the control soils.

(d) Many apparent losses of taxa observed in field monitoring are probably changes in the relative numbers of different groups in the community.

(e) Regular sampling is important because changes in community structures through the season and plant development, confer an added level of complexity to comparing GM vs. non-GM effects. Many of these effects are context-dependent and not systematic in character through the season.

(f) Arising from the case-by-case approach, specific targets for monitoring are selected, which have intrinsic and clear definitions of damage; however, there is a lack of monitoring activity, which is linked to a concept of damage to the system.

Lilley et al. (2006) recommended the establishment of long term monitoring to collect data on potential slowly accumulating effects, effects that become apparent when land-use conditions change, and scaling up effects after commercialization. The results of this monitoring will give a feed-back to improve the initial risk assessment.

While a considerable amount of research was carried out with MON810 Bt maize, other authors have investigated soils rhizosphere effects related to different GM plants:

Brusetti et al. (2004) used BT176 maize to study function and community structure of rhizosphere bacteria. The only differences detected were related to rhizosphere and bulk soil bacterial communities depending on the age of the maize plants examined with no influence of the genetic modification. Accordingly, the authors state that bacterial communities can be influenced by altering root exudates during host plant development, but not by the GM in question.

Devare et al. (2007) analysed the impact of Cry3Bb1 expressing MON863 on soil microbial activity and biomass during a 3-year field trial in the USA. The research group measured microbial biomass, nitrogen (N) mineralization potential, short-term nitrification rate, and respiration rate in rhizosphere and bulk soil samples collected. Sample size was designated to three replicate field plots just before planting, at anthesis, and at harvest in each year. The results of this study showed that there were clear seasonal effects on microbial biomass and activity in the field soils. This was apparent by the consistent changes in all analysed variables across years and sampling times. There were also some differences observed between bulk and rhizosphere soil parameters. However, there were no adverse effects of either the Bt or non-Bt maize (with insecticide treatment) compared to non-Bt controls. The data on microbial biomass and soil respiration suggests a stimulatory effect of the Bt genotype, particularly in comparison to the non-Bt isoline. In summary, the data suggest that cropping MON863 Bt maize is unlikely to adversely affect soil ecology during a period of at least 3 years.

We are not aware of any published data for direct adverse effects of HT plants to soil microorganisms. Indirect effects due to the use of the complementary herbicide (and root exudates containing herbicide residues) were reported on nitrogen fixing bacteria and pathogen fungi by Kremer et al. (2005) and Njiti et al. (2003) and are discussed in Section 4.7.

As stated before (e.g. EFSA, 2011d), effects of GM crops on soil microbial communities in the rhizosphere or on decaying plant material, depend more on the plant species, variety or age than whether they are genetically modified. Rearrangements in structural diversity and population abundance of non-target soil organisms occur frequently in the agricultural environment. They are typically associated with several sources of variation, caused by natural variability (e.g. soil heterogeneity, weather conditions) and agricultural practices (e.g. soil tillage, crop rotation, irrigation measures) and are thus not necessarily an indication of environmental harm.

Conclusions regarding effects on microbiota:

- Decaying plant material or root exudates containing products of GM plants may affect population size and activity of rhizosphere organisms.
- However, the results optained from field experiments suggests that products of GM plants, in particular Cry proteins, might only have transient negative effects on soil protozoa and microorganisms.
- The results reveal no or only some minor changes in soil microbial community structure.
- Consideration should be given to monitoring soil functions in order to detect potential long-term effects on important soil microbial functions (see e.g. BEETLE 2009).

4.5.6 Effects on Symbiotic NTOs

Interaction with symbiotic organisms has not been extensively studied with GM plants but there are some studies of on mycorrhiza. Mycorrhizal symbiosis provides a source of nutrients for both plants and microbes. It constitutes an important functional component of the soil plant system (Leyval et al., 2002). Effects of Bt maize (event Bt176) on mycorrhizal fungi have been reported by Turrini et al. (2004) and Castaldini et al. (2005) in microcosm and greenhouse experiments for one specific Bt maize event (Bt176). Significantly lower levels of mycorrhizal colonization of Bt maize roots was observed compared to non-Bt maize but no near isogenic control varariety was available in these experiments. Other reports are contradictory: de Vaufleury et al. (2007) did not find any differences in mycorrhizal colonization or infectivity between Bt (event MON810) and non-Bt maize in microcosms. Liu (2010) published an overview of research on the effects of GMPs on arbuscular mycorrhizal fungi, soil biochemical properties and microbial communities. It was concluded that data are still inconsistent on environmental effects and that present empirical data are incompatible. For HT crops, direct impact on symbiotic organisms has not been reported so far. However, there are data available on the effects of the herbicides applied to HT crops on rhizobia nitrogen fixing bacteria (see chapter 4.5.5).

Conclusions regarding effects symbiotic NTOs:

- There have been few studies of impacts on symbionts of GM plants
- It is still unclear whether some observed effects of Bt plants on symbiotic mycorrhyza are related to varietal effects or the genetic modification.
- Data so far suggests that these effects will not differ significantly from effects due to different conventional varieties and cropping systems.

4.5.7 NTO Species Selection for Testing GM Plants

There are several criteria suggested for species selection to conduct ERA for GM plants. The use of “surrogate species” is advocated, e.g., by Romeis et al. (2008a,b) in order to select appropriate species which are representative of their genera and/or of particular functional groups (including herbivory, pollination of cultivated and wild plants, predation and parasitism of pest organisms and decomposition of plant material) to serve as surrogates that can be tested under laboratory and/or field conditions. The concept of using surrogates is widely applied in regulatory toxicity testing, in monitoring the effects of environmental pollutants and in conservation biology to indicate the extent of anthropogenic influences. Surrogate species or guilds that are representative of different functional groups are known in most systems and appropriate surrogates can therefore be selected that are relevant to the agro-ecosystem of concern. Species selection would normally prioritize the functional role of these taxa, so that conclusions from the risk assessment address important processes and are broadly applicable. In addition, the problem formulation may consider species of anthropocentric significance, including those with special aesthetic or cultural value (e.g. the monarch butterfly in Northern America or the peacock butterfly in Europe) or species classified as threatened or endangered. The most effective surrogate taxa, including for example honeybees (*Apis mellifera*), are found in many different crops or regions. More specific, crop-associated species may be selected to represent an important genus (e.g. *Orius* spp.), and other taxa may be selected that are broadly representative of whole families (e.g. parasitic wasps of the Ichneumonidae) or orders (e.g. Coleoptera) that are known to be important for ecosystem services. The pest species that are screened for their sensitivity to the insecticidal protein during product development can also serve as surrogates for NTO’s. The familiarity with the species as a laboratory organism is deemed important in this approach.

The concept of using functional groups and anthropocentric values was also an important component of the Andow et al. (2006b) concept. A ‘key species’ selection process was developed in a stepwise approach to address effects on non-target organisms and biological diversity. The essential components include: 1) a risk endpoint selection process, 2) a process relying on risk hypotheses to guide the characterization of exposure, adverse effects and risk, and 3) a transparent prioritization of the selected species based on ecological characteristics for the specific system. Significant properties of the methodology include: 1) it relies on all available scientific information; 2) it relies first on qualitative information and methods and proceeds to quantitative approaches only as necessary; 3) it is structured in a way to overcome the lack of information, specifically addressing uncertainties, and 4) it considers the special needs of highly biodiverse countries. This stepwise selection is used to filter out 6 to 10 key species for GM crop/trait combinations which should then be examined in more detail.

4.5.8 EFSA GMO Panel Guidance

NTOs to be tested in ERA studies should be potentially exposed to the GM plant and its products in field conditions and good indicators of changes in the environment or of ecosystem functions. Problem formulation (see Figure 3) should identify the relevant NTOs potentially exposed to the GM plant or its products, taking into account the specific plant/trait combination and the receiving environment of the crop. An important criterion for the selection of NTOs to be assessed comes from the national environmental protection goals, and other legal EU frameworks provide guidance, in particular the Directive 2004/35/EC (EC, 2004) puts emphases on protected species and habitats as part of biodiversity. Other criteria for selection (see EFSA, 2010a) might be cultural value, conservation concern, or economic value (i.e. “anthropocentric functions”).

The EFSA GMO Panel (2010b) proposed criteria for NTOs selection, and provided advice on standardized testing methodology. The following requirements for NTO testing are described: Clear and objective protection goals for which assessment and measurement endpoints shall be developed, the need to initiate the scientific risk assessment by setting testable hypotheses, the criteria for appropriate selection of test species and ecological functional groups, appropriate laboratory and field studies to collect relevant NTO data, and the use of statistical techniques that should be an integral part of experimental design. A range of approaches and methodologies of ERA of NTOs is described in the current literature (EFSA, 2010b). Risk assessment approaches should be based on selection of functional groups and individual species within a tiered approach. The EFSA scientific opinion provides guidance to risk assessors for assessing potential effects of GM plants on NTOs, together with the rationale for data requirements in order to complete a comprehensive ERA for NTOs.

NTO species and ecological functions/services should be selected based on clear ecological criteria, from which focal species should be selected for testing. A theoretical framework is presented in Fig. 2 showing how to select focal species.

The species selection process should start with the analysis of the agroecosystem and the listing of the main arthropod species linked to it.

However, one should again notice that NTO species selection depends on crop*trait*region combination taking into account Table 2. From this initial selection a prioritization process is needed during which the applicant ends up with a decision on which NTO species will be used in the evaluation.

Additional criteria that might be of relevance in this selection of focal species are:

- the occurrence/presence of NTO (considering specific life stages) during the most likely period of exposure
- ecological significance of the species
- abundance of the species
- environmental sensitivity of certain NTOs (i.e. are their populations already threatened and thus more sensitive to additional pressures?)
- special attention should be paid and appropriate testing and sampling conducted for NTO species that have a life cycle and hence exposure extending over more than one crop growing season

Once the possible focal species are prioritized based on ecological criteria, the feasability and practicability of conducting tests on certain species can be used to further discriminate the species to be tested. Soil is a complex environment and it is challenging to employ laboratory bioassays to produce reliable predictions of what could happen to the below-ground ecosystem. However, such studies are currently approached by using species sensitivity distributions and by requiring specific tests for the main functions and life-forms. Biological indicators are often very dynamic and particularly sensitive to changes in soil conditions. Consequently, they are often used as markers of short-term changes in soil quality. Biological indicators include populations of micro-, meso- and if possible macro-organisms, or the study of its structure with multiple endpoints analysis addressing both diversity and processes.

Table 2: Functional Groups considered by EFSA (2010b) linked to GMP exposure through trophic interactions

Functional group		**Examples of taxonomic groups**
Herbivores		**Phloem-feeders:** aphids (*Hemiptera: Aphididae*), leafhoppers (e.g. *Hemiptera: Cicadellidae*), certain *Heteroptera* **Cell-content feeders:** thrips (*Thysanoptera: Thripidae*), spider mites (*Acarina*) and *Nematoda* (*Tylenchida: Meloidogynidae*) **Chewing:** leaf beetles (*Coleoptera: Chrysomelidae*), *Lepidoptera* larvae, *Diptera* larvae, grasshoppers (*Orthoptera Ensifera),* gastropods (*Mollusca, Gastropoda*)
Natural enemies	Predators	**Beetles:** *Coleoptera* (e.g. *Coccinellidae, Carabidae, Staphilinidae*) **Predatory bugs:** *Heteroptera* (e.g. *Nabidae, Anthocoridae*) **Predatory flies:** *Diptera* (e.g. *Syrphidae*) **Lacewings:** *Neuroptera* (e.g. *Chrysopidae, Hemerobidae*) **Thrips:** *Thysanoptera* (e.g. *Aeolothrips*) **Spiders & harvestmen:** *Araneae* and *Opiliones* **Mites:** Acarina (e.g. *Phytoseiidae*) **Nematoda** (e.g. *Mononchus* sp)
	Parasitoids	**Hymenoptera** (e.g. *Ichneumonidae*, *Braconidae*, *Aphelinidae*)
	Parasites & Pathogens	**Bacteria, fungi, viruses**
	Entomopathogenic organisms	***Nematoda*** (e.g. *Heterorhabditidae*, *Steinernematidae*), pathogenic microorganisms
	Pollinators	**Solitary and social bees** (*Hymenoptera: Apidae*), hover flies (*Diptera: Syrphidae*); Coleoptera (e.g. *Melyridae, Curculionidae, Scarabaeidae*)
	Decomposers	**Diptera larvae** (e.g. *Phoridae, Sciaridae*), Nematoda (e.g. *Rhabditidae, Dorylaimidae*), springtails (*Collembola*), mites (*Acarina*), earthworms (*Haplotaxida: Lumbricidae), Isopoda*, microorganisms
	Plant symbionts	**rhizobacteria, mycorrhiza**

In the categorisation of relevant NTO species, additional species of economic or aesthetic or cultural value, or species of conservational importance considered as threatened or endangered may also need to be included.

Conclusions regarding selection of NTO species for testing:

- Selection of NTO species is related to the crop*trait*region combination.
- Other selection criteria for the NTO species used in an evaluation include the feasibility of culturing the NTO, the coincidence of sensitive stages of its life cycle with exposure to the GMP or its products, and its ecological relevance in receiving environments.

4.6 Effects on Biogeochemical Processes

According to the EFSA guidance document (EFSA, 2010), there is a requirement to determine whether GM plants have adverse effects on biogeochemical processes. These could include alteration

- of climatic conditions (e.g. altered production of greenhouse gases),
- of mineralisation (e.g. root exudates changing the soil pH), and
- to soil nutrient cycling.

Changes in the abiotic environment caused by GM plants can have impacts on the biotic environment and changes to soil biota (caused by GM plants) can also result in changes to the abiotic environment, and thus there is interaction with soil NTOs as discussed in previous Sections.

4.6.1 Increased Production of Greenhouse Gases

“Global change” encompasses changes in atmospheric composition, climate, land cover and land use. These changes and their interactive effects on biological systems are on a worldwide scale (Scherm et al., 2000). Any living population in terrestrial or limnic environments including cultivated GM crops will be exposed to a complex of environmental alterations. One of most important causes for global change scenarios is increasing CO_2. Together with light, water, nutrients and temperature, CO_2 is an important factor in plant biomass production (Begon et al., 2005). There are a number of studies on the impact of green house gases on crops including several GM crops (e.g. Ryle and Powell, 1992, Traore et al., 2000, Pritchard et al., 2007, Wan et al., 2007, Lobell et al., 2008, Taub et al., 2008). By contrast the impact of plants on the production of green house gases (GMG) is in its infancy. Of particular interest is whether GM plants will contribute to climate change for example through increased production of green house gases emitted by the cultivation, production or processing of GM crops.

In general, intensification of agriculture has led to the use of fossil energy sources for agricultural practice and increased CO_2 emissions. Similarly, intensive high-yield agriculture is dependent on addition of fertilizers, especially synthetic N produced through a fossil fuel-consuming industrial process that converts abundant atmospheric N to available form for plants (Matson et al., 1997).

However, according to Brookes and Barfoot (2005) GM crops contributed to significantly reduced greenhouse gas emissions from agricultural practices. This reduction resulted from decreased fuel use, about 1.8 billion litres in the years 1996-2004, and additional soil carbon sequestration because of reduced ploughing or improved conservation tillage associated with biotech crops. According to the authors, this reduction was equivalent to eliminating more than 10 billion kg of carbon dioxide from the atmosphere in 2004. An increased adoption of soil conservation practices was also reported for the USA by Fernandez-Cornejo and Caswell (2006). In contrast, concerns were expressed that the production of HT soybean leads to environmental problems such as deforestation and soil degradation (Benbrook, 2005, Pengue, 2005).

Conclusions regarding effects biogeochemical processes:

- Literature data are currently limited with respect to impacts of GM crops on climate change.

- Information to date shows that where GM crops have reduced agrochemical usage and/or soil tillage then GHG emissions are reduced.

- If GM crops are associated with intensification of agriculture there may be higher use of fossil energy resources, and decline of the organic soil fraction, which might increase carbon dioxide release into the atmosphere. It is not likely that GM crops with potential relevance for Switzerland will adversely affect GHG emissions compared with current crops, though this will largely be influenced by how these crops are managed.

4.6.2 Increased Mineral Nutrient Erosion and Fertilizer Leaching

Decomposition and mineralization of organic matter are the main natural nitrogen and phosphorus sources in soils. There are concerns about potential detrimental effects on soil quality and function with respect to the increasing area of GM crops being cultivated worldwide (Motavalli et al., 2004). On the one hand soils could potentially be impacted by root exudates originating from the genetic modifications as GM plants produce recombinant metabolites throughout the season; on the other hand larger amounts of toxic compounds (e.g. Cry proteins) might be incorporated into soil after harvest. In addition, changes in compositional character of plant tissue could affect composition of plant necromass, degradation and nutrient cycling (Raubuch et al., 2007).

In addition Cry proteins could affect soil arthropods or soil microbes being involved in organic matter degradation (see chapter 4.5.1). Baumgarte and Tebbe (2005) found differences in microbial community structures in the rhizosphere of Bt maize compared to control treatments. However, the extent the alterations of microbial community structure due to Cry proteins was less than those determined by biological (e.g. age of plants) or physical factors (field heterogeneity). No differences were reported by Griffith et al. (2006) in soil microorganism communities by comparing conventional and Bt maize. Some differences were observed for protozoa depending on the year and experimental sites.

Increased lignin content in Bt maize residues could affect nitrogen mineralization (Saxena and Stotzky, 2001c). The authors found 33 to 97% higher lignin content in several Bt maize varieties. Additionally, Masoero et al. (1999) determined that two Bt maize varieties exhibited higher starch, higher lignin but lesser protein and nitrogen content. Flores et al. (2005) suggested that a higher C: N ratio of Bt maize would result in delayed N mineralization and could increase nitrogen fertilizer demand. Such additional applications of fast degradable organic or mineral fertilizer increase the likelihood of enhanced mineral fertilizer input into groundwater or of losses of fertilizer by surface runoff to water streams in vicinity of the fields.

In theory, higher lignin concentrations of Bt maize residues could lead to slower degradation of organic plant residues and an enrichment of organic matter in soils. This would be particularly important if Bt maize was be cultivated continuously in organically depleted fields. Under such conditions losses of mineral fertilizers from soils would be reduced. However, altered lignin content in maize varieties is not an effect attributed to the insertion of the transgene (see chapter 4.5.2), but from the genetic background of the maize varieties under consideration (Fernie et al., 2006; Griffiths et al., 2007; Lehman et al., 2008b, 2010; Zurbrügg et al., 2010; Yanni et al., 2011). For example, the total lignin content in the roots and leaves of maize MON 88017 is slightly higher (7 %) or similar, respectively, compared with the near-isogenic counterpart (Poerschmann et al., 2008; Zurbrügg et al., 2010). In a litter bag study, Zurbrügg et al. (2010) found that leaf litter from maize MON 88017 is quickly degraded. According to EFSA (2011d) the degradation did not differ from the near-isogenic counterpart, but varied among conventional maize varieties. Compositional plant properties including lignin contents among conventional maize varieties differed more than between maize MON 88017 and its near-isogenic counterpart, with maize MON 88017 falling within the variation found in conventional maize varieties (see also Lehman et al., 2008a, 2010 for Cry3Bb1-expressing maize events).

Direct input of pollen and other parts of Bt maize plants into headwater streams nearby to maize fields cultivated with Bt maize in the Midwest of USA was investigated by Rosi-Marshall et al. (2007). They found evidence for transport of the Bt containing maize residues downstream in the water bodies. However, with respect to the breakdown rates of Bt containing plant litter, no differences were found between Bt and non-Bt containing litter. In laboratory feeding trials with aquatic insects (*Helicopsyche borealis*) decreased growth rates and in one case an increased mortality was observed with high amounts of Bt maize pollen. Aquatic ecosystems were also studied by Douville et al. (2007). The group spiked surface water and sediment of a surface water body in Canada with genomic maize DNA containing the cry1Ab gene. At different times in a season samples from water and sediment were collected and tested for cry1Ab residues. The gene was still detected 40 days after introduction in clay and sand-rich sediment. Persistence of the genes was significantly higher in sediments than in the open water. Potential effects of Bt-proteins on NTO in aquatic ecosystems are discussed in chapter 4.5.1. However, the is no indication for any effect of Bt expressing GMP on increased mineral nutrient erosion and fertilizer leaching.

Some proteins expressed in herbicide tolerant plants also occur in conventional plants (e.g. EPSPS) and /or in microorganisms (e.g. pat or bar) (Busse et al., 2001) and are not known to act differently to the naturally occurring ones. Because the CP4 EPSPS protein of GMP is homologous to the EPSPS proteins found in plants and microorganisms (CERA, 2010), it is unlikely that it will affect microbial communities (EFSA, 2011d) and hence biogeochemical processes adversely. Likewise, the expression of the newly introduced traits in GM plants, which are naturally occurring in the soil environment, are not expected to alter the natural interactions of maize plants with the abiotic environment.

Some herbicides can remain in soil and affect micro-biota and so applications of the non-selective herbicides used on herbicide tolerant crops may affect the biotic and abiotic soil environment. However, removal of ground cover plants may increase the likelihood for losses of nutrients by surface run-off and soil erosion which in turn could lead to an increased risk of eutrophication of water courses in agricultural landscapes (Haney et al., 2000, 2002). This kind of risk would depend on the herbicide management as well as the chemicals used.

Another potentially adverse aspect of glyphosate usage on nutrient availability is reported by Eker et al. (2006). Glyphosate applications in low doses (~ 6% of recommended dosage) to non-target (glyphosate-sensitive) sunflowers (*Helianthus annuus*) resulted in significantly reduced root uptake of Fe and Mn. Also root-to-shoot translocation of the cationic minerals was almost completely inhibited potentially leading to severe impairments in Fe and Mn nutrition of non-target plants. Due to the chelating effect of glyphosate poorly soluble glyphosate-metal complexes may be formed in plants as well as in the rhizosphere reducing the availability of these two nutrients. US farmers are recommended to add Mn to fields cultivated with glyphosate-tolerant crops.

Conclusions regarding mineral nutrient erosion and fertilizer leaching:

- Data are limited on impacts of GM crops on soil mineral and organic nutrients and no harmful effects have been reported that are additional to those found in conventional crop production.

- Intensive and repeated cultivation of Bt and HT crops may impact soil organisms involved in nutrient cycling but it is not clear whether these affects will effect soil nutrient status and are any different from those associated with some conventional practices

- An indirect effect of glyphosate as a chelating agent of Fe and Mn availability is reported that potentially might cause increasing nutrient deficits.

- It is considered unlikely that the current GM crops would have adverse effects on soils if cultivated in Switzerland.

4.6.3 Persistance and Fate of GMP Expressed Products in Soil

Icoz and Stotzky (2008) have published an extensive review on the fate and behaviour of Bt proteins in soils. They report very different results with respect to the persistence of Cry-proteins in soils. Half-lives of cry1Ab protein range from 1.6 days (Sims and Holden, 1996) up to 34 days (Wang et al., 2006b). Also long-term persistence of cry proteins in soils is variable. Cry1Ab proteins in low concentrations were detected up to 56 days (Donegan et al., 1995) or up to 234 days (Tapp and Stotzky, 1998) or up to 180 to 350 days in residues of Bt maize (Saxena and Stotzky, 2002).

Gruber et al. (2011a) investigated the fate of Cry1Ab in soil under long-term Bt-maize cultivation in an experimental field trial performed over nine growing seasons on four South German field sites cultivated with MON810 and its near isogenic non Bt-maize variety. The Cry1Ab protein was never detected in soil sampled in the spring before the next farming season at any of the four experimental sites. There was no evidence for accumulation or persistence of Cry1Ab protein in different soils under long-term Bt-maize cultivation.

These reported differences in half-life and persistence of Bt proteins in soil may be a result of the specific chemical and physical conditions in the soils. Of special importance are pH, clay content and type and electrokinetic charge of external clay surfaces (Pagel-Wieder et al., 2007, Blackwood and Buyer, 2004). Icoz and Stotzky (2007) found differences in Cry3Bb1 persistence in soils depending on the type of the predominant clay minerals. The protein was found for a short period of 21 days in the presence of montmorillonite. If kaolinite was amended, the protein was detectable for 40 days. However, if pH was adjusted to 7 the protein was only found for up to 20 days in kaolinite.

Pagel-Wieder et al. (2007) have chosen an approach to elucidate the interdependence between chemical and physical soil properties and Cry protein persistence. The authors tested the adsorption of Cry1Ab protein at Na-montmorillonite and were able to perform adsorption kinetics of Cry protein and clay. The best adsorption per unit weight of the protein was detected with high protein and low but highly dispersed clay concentrations. With higher clay concentrations the minerals clumped leading to lower surface charge or binding sites exhibiting relatively decreased adsorption capacity. Additionally, with lower soil pH binding of Cry 1Ab to clay surfaces was improved. About 10% of the bound Cry proteins could be recovered by washing. A variability of shape confirmations is known for Cry proteins (e.g. folded/unfolded shapes, oligomerized/non-oligomerized forms) according to Schnepf et al. (1998), Bravo et al. (2004) and Rausell et al. (2004). It is still unclear whether there is a relationship between protein shape and soil adsorption.

Madlinger et al. (2011) studied the processes governing the fate and bioavailability of the expressed transgenic Cry proteins in soils in laboratory experiments. Experimental and modeling evidence was provided by the authors that the surface heterogeneity of soil SiO_2 particles modulated electrostatic attraction, leading to a fraction of adsorption sites with slow Cry1Ab desorption kinetics. Desorption rates from these sites increased upon increasing the solution pH. In toxicity bioassays, Madlinger et al. (2011) demonstrated that Cry1Ab retained insecticidal activity when adsorbed to SiO_2, suggesting high protein conformational stability during adsorption desorption cycles. The authors concluded that models predicting Cry1A protein adsorption in soils need to account for combined effects of the nonuniform protein surface charge distribution and of sorbent surface heterogeneity. This means that adsorption to polar, charged surfaces in soils does not inactivate the Cry1A protein.

In addition the active ingredients of non-selective herbicides are at least partially bound to soil particles. For glyphosate a rapid adsorption in soils is reported (Goldsborough and Brown, 1993). Nevertheless, some publications emphasize transport processes of the negatively charged glyphosate in soils. Those transports are depending on structural and chemical soil characteristics like clay content or iron vice versa phosphate availability (Gimsing and Borggard, 2002, Borggard and Gimsing, 2008). Mobility of glyphosate is increased to a small extent if pH is high. Glufosinate will also be bound by soil particles; however the efficiency of binding is moisture dependent (Gallina and Stephenson, 1992). According to EFSA (2011d) a large number of authors have claimed that some of the herbicidal active substances used on GMHT crops (e.g. glyphosate) have reduced environmental impacts compared with those applied on their conventional counterparts (Nelson and Bullock, 2003; Peterson and Hulting, 2004; Brimner et al., 2005; Brookes and Barfoot, 2006; Leroux et al., 2006; Kleter et al., 2007; Bonny, 2008, 2011; Devos et al., 2008; Arregui et al., 2010; Mamy et al., 2010). The environmental impact indices used for these calculations are generally based on residual, persistence and ecotoxicity characteristics, and do not relate to the efficacy and hence the biodiversity impact of herbicides. The ecotoxicological effects of herbicides fall under the Plant Pesticide Directive 91/414/EC and will not be further discussed in this report as these a direct effects of the herbicide and not of the HT GMP. Indirect effect on biodiversity of the use of non-selective herbicide in GM HT crops are discussed in chapter 4.7.

In general, if binding to the surfaces of soil minerals of GM plant (directly or indirectly) related metabolites occurs, this could lead to a reduction of the exchange capacity of soils. Cation exchange capacity (CEC) of soils is responsible for preventing the risk of cationic nutrients being transported through the root zone into lower soil horizons or into the groundwater. This beneficial effect of nutrient retention would be decreased by binding of large amounts of Bt proteins to these soil particle sites. Additionally, the negatively charged clay surfaces play an important role for binding H^+-cations. If the H^+-binding was affected by Bt protein buffering then the neutralizing of high H^+-concentration in soil would be altered, and lower soil pH. This theoretical possibility has not been reported.

Effects of GMP on biogeochemical processes e.g. via microbial communities, which are especially expected in their rhizosphere or on decaying plant material, depend more on their species, variety or age than whether they are genetically modified. Rearrangements in structural diversity and population abundance of non-target soil organisms occur frequently in the agricultural environment. They are typically associated with several sources of variation, caused by natural variability (e.g. soil heterogeneity, weather conditions) and agricultural practices (e.g. soil tillage, crop rotation, crop type and variety, irrigation measures) and are thus not necessarily an indication of environmental harm (EFSA, 2011d).

In summary, there is no indication for the accumulation of GMP expressed products in soil which could raise concerns for biogeochemical prosesses. With respect to biogeochemical processes and effects on NTO (see chapter 4.5) a number of studies (reviewed by Widmer, 2007; Filion, 2008; Icoz and Stotzky, 2008) were performed under laboratory, glasshouse or field conditions covering a large array of classical and more recent analytical tools. These studies revealed only some minor changes in soil microbial community structure with Bt-maize compared to non-Bt-maize (Blackwood and Buyer, 2004; Brusetti et al., 2004; Griffiths et al., 2006; Mulder et al., 2006) or generally show no adverse effects of the Cry1Ab protein released by Bt-maize in root exudates or from biomass incorporated into soil microorganisms or microorganism-mediated processes (Saxena and Stotzky, 2001a, Flores et al., 2005, Anonymous, 2006, Hönemann et al., 2008, 2009, Icoz et al., 2008). Miethling-Graff et al. (2010) did not detect any significant differences between the rhizosphere bacterial community structure of Cry3Bb1 expressing maize MON 88017 compared with the near-isogenic counterpart and two conventional maize varieties in their 3-year field study in Germany.

Conclusions regarding persistence and fate of GMP expressed products in soil:

- Several studies on potential effects have been performed under laboratory, glasshouse or field conditions covering a large array of classical and more recent analytical tools.
- There is no indication for the accumulation of GMP expressed products in soil.
- There are no indications of adverse effects on soil nutrient cycling or on soil functions due to GMPs.

4.7 Impacts of the Specific Cultivation, Management and Harvesting Techniques

In the EU, Directive 2001/18 specifically requires that the environmental impacts of any specific cultivation techniques or measures associated with the GM crop are considered. Thus, as with the introduction of any new crops, the cultivation of GM crops (e.g. pest resistant or herbicide tolerant crops) may alter current management regimes and may introduce new cropping techniques (Champion et al., 2003, Hayes et al., 2004, Collier and Mullins, 2011). Any environmental impacts of these changes should be compared with current “standard” practices to determine whether any adverse environmental effects are likely to occur.

4.7.1 Herbicide Tolerant Crops

Genes conferring tolerance against specific non-selective herbicides are currently among the most common traits introduced into GM plants. Today, there are several different HT systems available on the market: introduced GM tolerance (e.g. ALS, glyphosate and glufosinate-ammonium tolerance) and non-GM technologies (e.g. Imidazolinone – Clearfield technology, Atrazine). Soil bacteria with a resistance to glyphosate and glufosinate are widespread and naturally occurring in the environment. Inactivation of glyphosate and the expression of glyphosate tolerant EPSPS synthases were transferred from bacteria to plants.

Glyphosate and glufosinate are broad-spectrum systemic herbicides used for the control of annual and many perennial weeds (Duke and Powles, 2008b), but with no soil acting or little residual properties.

According to Directive 91/414/EEC (recently substituted by regulation 1107/2009/EC), a plant protection product may only be authorised in the EU if its active substances are listed in Annex I of Directive 91/414/EEC, and if its application has – inter alia – no harmful effect on human or animal health and no unacceptable influence on the environment. Contrary to the overall evaluation of the environmental safety by the EU competent authorities in the field of plant pesticide registration, Schütte and Mertens (2010) concluded that the direct use of glyphosate and glyphosate-based herbicides negatively affect soil and aquatic organisms and plant health. The authors claim that glyphosate, interacts with certain fungal crop diseases and endangers amphibians. Glyphosate and its degradation product, aminomethylphosphonate (AMPA), residues are not usually detected in high levels in ground or surface water in areas where glyphosate is used extensively. In addition both glyphosate and AMPA are considered to be much more toxicologically and environmentally benign than most of the alternative herbicides (see e.g. Cerdeira and Duke, 2010). The evaluation of active substances and plant protection products according to Directive 91/414/EEC comprises a comprehensive toxicological and ecotoxicological assessment including the assessment of effects on soil micro-organisms. The German Competent Authority (BVL) has considered the results of these evaluations for glyphosate and for the use of glyphosate on genetically modified herbicide-tolerant sugar beet and fodder beet. Based on its assessment, the German Competent Authority has reached the conclusion that the direct impacts of weed control in glyphosate tolerant sugar beet on the environment (including non-target animals and soil microorganisms) will be within the range of the environmental impacts of weed control methods that are currently used in sugar beet in the EU.

In the EU, glyphosate is currently used in conventional cropping and can be used pre-emergence of annual crops and for controlling emerged weeds in seedbeds and stubbles prior to cultivation. Both herbicides are also used as preharvest dessicants of annual crops and around perennial crops. In addition, in some situations, glyphosate and glufosinate can be applied in an emerged crop as a band application between crop rows (Monsanto, 2007a).

Herbicide regimes in GMHT crops: the broad spectrum herbicide is applied post-crop emergence to established weeds providing high levels of weed control and little or no to injury the crop. Theoretically, the biotechnology-based weed management strategy enables delaying the post-emergence application of broad-spectrum post-weed management, compared to non-GMHT maize (Gianessi, 2005; Cerdeira and Duke, 2006, 2007, 2010). Because the efficacy of glyphosate at controlling weeds is less dependent on weed size, glyphosate can be used up to a later growth stage for weeds. Therefore, the biotechnology-based weed management strategy offers a greater flexibility in timing of weed management. However, the control of larger and perennial weeds might require higher application rates (Monsanto, 2007a). Experimental research has shown that a single post-emergence application of glyphosate alone at the recommended application rates might be inadequate (Gianessi et al., 2002, Gower et al., 2003, Parker et al., 2006). If the first single treatment is applied early, later-emerging weeds will be unaffected. These weeds can reduce crop yield by competing for resources and might set seed that replenishes the seed bank, or survive vegetatively until the following season, increasing weed pressure in subsequent years (e.g. Myers et al., 2005). Thus, in the absence of pre-emergent residual herbicides, a sequential application of glyphosate might be needed to control later emerging weeds. Recommended strategies to avoid weed reinfestation involve the use of two post-emergence applications of glyphosate (Gower et al., 2003). In this respect, Monsanto recommends using glyphosate at dose rates ranging between 1440 and 2160 g/ha ai (active ingredient) in two applications (Monsanto, 2007a) and dose rates ranging between 1800 and 2160 g/ha ai in two applications (Monsanto, 2007b). In a field trial with maize NK603 in Czech Republic, optimum herbicide efficacy was provided by a split application of glyphosate (1080 + 1080 g/ha ai) (Soukup et al., 2008).

Delaying the first single treatment with glyphosate can lead to yield reductions due to an extended period of early weed competition (Gower et al., 2002, 2003; Champion et al., 2003; Cox et al., 2006). To limit early-season competition and ultimately maize yield losses, and to eliminate the need for a second post-herbicide application, the use of pre-emergence residual conventional herbicides followed by one delayed post-emergence glyphosate spray has been suggested (Thomas et al., 2004; Parker et al., 2006). In this situation, the application rates of glyphosate recommended by Monsanto are within the range of 720 to 1440 g/ha ai (Monsanto, 2007a).

In regions where early post-emergence herbicides are predominantly used, a single application of glyphosate in mixtures with other post-emergence herbicides with residual activity is considered effective for glyphosate tolerant maize (e.g. Gianessi, 2008; Soukup et al., 2008). This will eliminate early-season weed competition and will control the weeds that are not exposed to glyphosate (Thomas et al., 2004; Dill, 2005; Tharp et al., 2004; Grichar and Minton, 2006; Parker et al., 2006; Young, 2006; Zuver et al., 2006). Based on field studies conducted at 35 sites throughout the north-central US, Gower et al. (2003) concluded that the optimum timing for the glyphosate application to avoid maize yield loss is when weeds are less than 10 cm in height, no later than 23 days after maize planting, and when maize growth was not more advanced than the 4 leaf stage. In that case, the recommended glyphosate application rates are within the range of 720 to 1080 g/ha ai (Monsanto, 2007a) and dose rates of 1080 g/ha ai when mixed with another herbicide with residual activity (Monsanto, 2007b).

In the Technology Use Guide developed by Monsanto for the use of Roundup PRO2 in France (Monsanto, 2007a), the applicant recommends the following application patterns and rates for the use of glyphosate on maize NK603: i) a sequential application pre- and post-emergence at rates ranging from 720 to 1440 g/ha ai; ii) two applications post-emergence at rates ranging between 720 and 1080 g/ha ai each.; iii) single application post-emergence of a mixture (Roundup PRO2 + residual activity) at rates around 1080 g/ha ai. The maximum annual usage dose is set at 2880 g/ha ai.

In the Technology Use Guide developed by Monsanto for the use of Roundup Ready in Spain (Monsanto, 2007b), the applicant recommends the following application patterns and rates for the use of glyphosate on maize NK603: i) a sequential application pre-emergence with a selective herbicide with residual activity and post-emergence with Roundup Ready at rates of 1080 g/ha ai; ii) two applications post-emergence at rates ranging between from 900 and 1080 g/ha ai each; iii) single application post-emergence of a mixture (Roundup Ready + residual activity) at rates around 1080 g/ha ai. The maximum annual usage dose is set at 2880 g /ha ai.

A diversity of weed management regimes is likely to be used in the different agricultural regions across the EU when GMHT crops are cultivated. EU countries show considerable variation in herbicide use in crops such as maize, sugar beet, soya and oilseed rape depending on weed species (including crop volunteers), meteorological and agro-environmental conditions, farming systems (including weed resistance management, rotation systems), economics, and in farmers' behaviour. Moreover, herbicide regimes are dependent on crop type and on weed species and weed populations as weeds have different life cycles and not all stages are equally susceptible to glyphosate (e.g. Norsworthy et al., 2001; Soukup et al., 2008, Albajes et al., 2009). This would mean that the locally adopted herbicide regimes and cultivation management (including conservation tillage) for GMHT crops will take into account all these factors. Therefore, it is anticipated that herbicide regimes containing glyphosate will vary according local conditions and hence will differ in numbers of applications (single vs. sequential), doses, timing of application and the use of residual herbicides in association with glyphosate.

Interplay between Directive 2001/18/EC and Directive 91/414/EEC

The registration and use of herbicidal active substances in formulations in the EU was covered by Directive 91/414/EEC (which is now replaced by Regulation (EC) No 1107/2009) as operated by individual Member States and both are relevant for the risk assessment of GMHT crops and their associated weed control management practices (EC, 2008, EFSA, 2008a, Ehlers, 2011). Where GMHT plants rely on specific herbicides as an integral part of a weed management strategy, an environmental risk assessment must also consider their potential impact on biodiversity under Directive 2001/18/EC. In the current legislation governing the registration of plant protection products in Europe, the environmental risk assessment of pesticides includes an assessment of impacts on certain non-target organisms (such as fish, Daphnia, algae, birds, mammals, earthworms, bees and beneficial arthropods and non-target plants) and studies of residual activities in soil and water (cf., environmental fate) (Streloke, 2011).

As already stated in chapter 4.5.1 (direct toxic effects on NTO), a large number of authors have claimed that some of the herbicidal active substances used on GMHT crops (e.g., glyphosate) have reduced environmental impacts compared with those applied on their conventional counterparts (Nelson and Bullock, 2003, Peterson and Hulting, 2004, Brimner et al., 2005, Brookes and Barfoot, 2006, Leroux et al., 2006, Kleter et al., 2007, Bonny, 2008, 2011, Devos et al., 2008, Arregui et al., 2010, Mamy et al., 2010, Stewart et al., 2011). Environmental impact indices used for these calculations are generally based on residual, persistence and ecotoxicity characteristics. However, they do not relate to the efficacy and hence the biodiversity impact of herbicides (e.g., van der Werf, 1996; Reus et al., 2002). As concluded by EFSA (2012a), the environmental risk assessment under Directive 91/414/EEC does not include studies of impacts on biodiversity within crops and changes in agro-ecosystems, which are required under Directive 2001/18/EC in relation to GM crops. Due to these different legal requirements in the EU, a herbicide used on a GMHT crop is assessed differently from the same herbicide used on non-GMHT crops (e.g., imidazolinone tolerant crops) and conventional crops (Chassy et al., 2003, Sweet and Bartsch 2011). The assessment of GMHT crop regimes includes evaluating potential effects on farmland biodiversity, while this is not a requirement for non-GM crop herbicide regimes (ACRE, 2007b, Morris, 2007, Sanvido et al., 2007, 2011a,c, Ehlers, 2011). However, though an assessment of indirect effects of herbicidal active substances on biodiversity was not required for the risk assessment of pesticides under Directive 91/414/EEC, the new Regulation (EC) No 1107/2009, concerning the placing of plant protection products on the market, explicitly mentions biodiversity as a protection goal (Streloke, 2011). Moreover, Directive 2009/128/EC aims to strike a new balance between food security and the support of biodiversity by promoting the sustainable use of pesticides.

Weeds have numerous interactions with other organisms and, in turn, some of these interactions can have direct, either negative or positive, effects on the functioning of the agro-ecosystem (Petit et al., 2010). It has long been recognized that the widespread use of herbicides in agriculture has resulted in serious declines in both plant and animal diversity in many farming areas (Krebs et al, 1999, Chamberlain et al., 2007, Robinson et al., 2002). Concern has been expressed that GMHT crops, through the in-crop use of very effective broad-spectrum herbicides, will further deplete biodiversity in farmland.

Factors affecting impacts in Europe

Extensive research has shown that impacts on the environment depend upon a wide range of baselines and on agronomic and environmental factors, which vary from region to region and from season to season. For example, Firbank et al. (2003b) commented following the UK Farm Scale Evaluations that major sources of variation in potential impacts would arise from probable future changes in agricultural practice such as herbicide regimes, tillage systems and crop rotations and from possible long-term interactions between weed and invertebrate populations. Most importantly, they stressed that the impact on biodiversity depends greatly upon the management of crops, rotations, and upon the provision of forage and habitat resources across the entire farmed landscape. Included in crop management is the dose being applied and the time and frequency of applications of the specific non-selective and other herbicides (Champion et al., 2003). Timing of application is particularly important, since with broad-spectrum herbicides sprays are often delayed until a later plant growth stage than is the case with the more selective herbicides associated with conventional crops. The higher mortality of larger (reproductive) individual weeds caused by the later herbicide application in GMHT crops (Heard et al., 2003b) tends to reduce the persistence of plant populations in the farmed landscape and reduce seed densities and in turn emerged plants. This loss of food resources is likely to cause reductions in the abundance of key invertebrate groups (Hawes et al., 2003) and of species at higher trophic levels, such as farmland birds.

All of the factors above will vary from region to region, from Member State to Member State, and from season to season. They depend not only on the nature of the particular receiving environment, but on weed pressure, soil type and climatic conditions. For these reasons, there are considerable challenges to the drawing of meaningful conclusions on the environmental consequences of the use of herbicides that includes consideration of every issue involved, over the full range of possible parameters that may be varied in the management of the GMHT crops, and the full range of receiving environments within Europe.

The focus of the environmental risk assessment should be on regions where the GM crop will be cultivated. However the environmental variation between arable ecosystems within regions is often as large as that between Member States. Two additional factors hamper the accuracy of estimates of impacts attempted at the European scale. First, individual Member States operate different regulations concerning certain aspects of conventional herbicide management applied to potential non-GM comparator crops, so there are difficulties in the quantification or establishment of detailed baselines in such dynamic situations for comparative analysis (Champion et al., 2003; Firbank et al., 2003a; Heard et al., 2005). Second, each EU Member State will have different baselines for the impact of current farming practices on the environment. These will influence Member State's policies on what are termed variously: environmental stewardship for farmland, biodiversity action plans, integrated pest management (IPM), good farming practice, etc. As reviewed in the EU resesearch project ENDURE, pesticides could potentially be reduced by using other means of pest control such as the choice of varieties including genetically modified hybrids, cultural control including crop rotation, biological control, optimized application techniques for chemicals, and the development of more specific treatments (Meissle et al., 2010). The authors concluded, however, that restrictions in the availability of alternative pest control measures, farm organization, and the training and knowledge of farmers need to be overcome before the adoption of environmentally friendly pest control strategies can reduce chemical pesticides in an economically competitive way. IPM approaches are needed to address the complex of several problems be tackled simultaneously. There is a link between different control measures. Pest and weed control needs to be seen in the context of the cropping system and on a regional scale (Meissle et al., 2010).

Environmental impacts of herbicide regimes used in GMHT cropping systems
GMHT plants tolerant to glyphosate-based herbicides allow these herbicides to be directly applied to the growing crop to give effective control of weeds (Beckie et al., 2006, Soukup et al., 2008). There is extensive literature on the range of effects of the use of glyphosate and its associated management in glyphosate tolerant crops (Cerdeira and Duke, 2006). For example in Western Canada, a reduction in the total number of chemical applications over a 3-year period was reported, resulting in a decrease of herbicide active ingredient being applied to farmland of nearly 1.3 million kg annually. Fewer tillage passes over the survey period were reported, improving moisture conservation, decreasing soil erosion and contributing to carbon sequestration in annual cropland (Smyth et al., 2011).

Some studies have focused specifically on the impacts of glyphosate associated with GMHT crops in Europe, though there is information on GMHT maize from Albajes et al. (2007, 2009) and Soukup et al. (2008, 2011). In addition, projects such as the project on botanical and rotational implications of GM herbicide tolerance in winter oilseed rape and sugar beet (BRIGHT) (Sweet 2003, Sweet et al., 2004, 2006) and the Farm Scale Evaluations (FSE) (Firbank et al., 2003a,b, 2006, Bohan et al., 2005, Champion, 2011) in the United Kingdom and the NERI study in Denmark (e.g., Strandberg and Pedersen, 2002, Strandberg et al., 2005) have studied GMHT sugar beet and fodder beet treated with glyphosate. Also, there is information from the FSE on GMHT forage maize treated with glufosinate. Additionally there are some other studies of herbicide tolerant crops in European countries that have compared conventional production systems with GMHT systems (Madsen and Jensen, 1995, Bückmann et al., 2000, Coyette et al., 2002, Soukup et al., 2008, Verschwele and Mülleder, 2008, Albajes et al., 2008, 2009, 2010, 2011, Szekeres et al., 2008, Thieme, 2010, Verschwele, 2011, Pálinkás et al., 2012).

The FSE remain one of the largest ecological experiments ever carried out to answer scientific questions about what impact the introduction of newly produced GM crops might have on farmland wildlife. It concluded that the scientific impacts of these crops and their environmental effects were due to the management of the herbicide tolerant trait which they included (Champion, 2011).

Adverse effects on biodiversity of the management of glyphosate tolerant crops have been reported in several experimental studies in Europe (Andow, 2003, Brooks et al., 2003, Hawes et al., 2003, Heard et al., 2003a,b, Lutman et al., 2008, Squire et al., 2009). Recently in the USA the loss of weedy host plants of Monarch Butterfly in agricultural fields due to glyphosate use has been indicated as causing reductions inpopulations of Monarchs (Brower et al., 2011a, Pleasants et al., 2012; see also critical remarks by Davies (2011) and the response by Brower et al., 2011b). Although cumulative effects on biodiversity due to the continuous cultivation of a GMHT crop have been predicted (Heard et al., 2005), such effects remain to be confirmed by field data. By contrast, the FSE reported a generally greater abundance of biodiversity in GMHT maize treated with glufosinate, than in conventionally treated maize. Perry et al. (2004) reported that these conclusions for maize would likely be affected in degree but not in direction by the withdrawal of atrazine from conventional herbicide management. Following the FSE, the advice from the UK Advisory Committee for Releases to the Environment (ACRE) was that biodiversity was likely to be reduced by the herbicide management of glyphosate tolerant sugar beet and glufosinate tolerant oilseed rape and should not be commercially cultivated using these management regimes. The FSE indicated that GMHT maize (glufosinate tolerant) tested in FSE could be cultivated in the UK using the regimes used in the FSE, because this would not result in adverse effects, as defined and assessed by criteria specified in Directive 2001/18/EC. This advice was adopted by the Department of Environment and Rural Affairs in the UK (DEFRA, 2005). Similarly in its early scientific opinions on maize Bt11 and 1507, the EFSA GMO Panel considered that the use of glufosinate ammonium in the cultivation of these two maize events is not likely to give an increased impact on biodiversity in most situations (EFSA, 2005a,b).

It is well known that weed community shifts will affect food resources leading to food chain effects on weed-associated fauna and higher trophic levels (Heard, 2005). Schütte and Mertens (2010) concluded that glyphosate resistant sugar beet management will have negative effects on biodiversity. The abundance and composition of weed flora was strongly altered by differential herbicide treatments in a four year field trail in Spain (Albajes et al., 2009). Several groups of arthropods responded to the weed changes but in variable directions. Whereas leafhoppers and aphids were more abundant on herbicide-treated plots, the contrary was found for phytophagous thrips. Among predators, *Orius* sp., spiders and trombidids were more abundant on treated plots, whereas nabids and carabids were more abundant in untreated plots, and more carabids and spiders were caught in pitfall traps. Among parasitoids, ichneumonids were more abundant in untreated plots and mymarids in treated plots. According to Bigler and Albajes (2011) many articles show that maintaining some weed enhances arthropod densities and natural biological control: GMHT crops may increase adoption of minimum and no tillage systems with possible effects on weeds and arthropods. Some weed management systems with GMHT plants also have the potential to alter plant diversity in crops in favor of conservation of biological control.

The above studies have confirmed that effects on weed populations, and hence biodiversity, are very dependant on the management of the herbicides in GMHT and conventional crop production systems and on the herbicides used in both systems. In some circumstances, such as with high dosage or repeated applications, the use of glyphosate with GMHT crops will more than likely result in reductions in botanical diversity in fields which in turn might adversely affect food chains and webs.

Environmental risk assessment must recognise that farming systems are highly dynamic, and that the introduction of widespread broad-spectrum herbicide systems may lead to substantial changes in management and biodiversity. There is now increasing knowledge from cultivation of GMHT crops worldwide that continuous and repeated application of glyphosate is causing changes in weed flora and development of more resistant or tolerant weeds (Fernandez-Cornejo and Caswell, 2006). Weed shifts have initially been discussed by Marshall et al. (2003) and noted by Owen and Zelaya (2005). Powles (2008) has observed the early development of resistance in some weed species and its effect in inducing modification of farmers' weed management through intensification of herbicide usage and subsequent adverse environmental effects. Herbicide resistance in weeds is not a topic or concern specifically focused upon the relatively recent introduction and adoption of GM crops, since the first case of herbicide resistance in weeds was reported in the scientific literature in 1970 (Owen 2010). However, if farmers were to repeatedly grow two or three successive glyphosate tolerant crops (such as sugar beet, maize, and soybeans) in their rotations, this would further increase selection pressure for such weeds. Maintaining diversity in crop rotation (HT and non-HT crops) coupled with the use of herbicides with different modes of action, is an important factor in lessening this risk (Duke and Powles, 2009, McGinnis et al., 2010). Currently, 21 weed species have evolved glyphosate resistant populations globally and twelve glyphosate resistant weed species have been identified in the USA, most of which evolved resistance to glyphosate in GMHT cropping systems (Beckie, 2011, Heap, 2011). The basis for resistance has been attributed to altered EPSPS target site, reduced translocation or cellular transport to the symplast, and sequestration in the vacuole (reviewed by Powles, 2008, Powles and Yu, 2010, Ge et al., 2010, Beckie, 2011, Shaner et al., 2011, Vila-Aiub et al., 2011). The problem of glyphosate resistant weeds is exacerbated by the fact that new resistance mechanisms such as gene amplification are being found (e.g. Gaines et al., 2010). Moreover, the evolution of multiple and cross resistances to herbicides is becoming increasingly more common (Heap, 2011). The overreliance on glyphosate to control herbicide resistant weeds contributed to the evolution of multiple resistances in populations (e.g. two or more resistance mechanisms) as a consequence of sequential selection and pollen flow, such as in glyphosate resistant *Lolium* spp. in Australia and South Africa (Neve et al., 2004, Yu et al., 2007, Preston et al., 2009, Preston, 2010) and in *A. palmeri* in cotton fields in southern USA (Culpepper et al., 2010). Multiple resistances to ALS-inhibiting herbicides and glyphosate are reported in horseweed (*Conyza canadensis*) (Davis et al., 2009).

It is important to note that glyphosate does not 'cause' weeds to evolve resistance per se, but rather how it is used that leads weeds to evolve resistance (Owen, 2011, Owen et al., 2011, Wilson et al., 2011). Evidence from the USA confirms that, where there is very intense glyphosate selection (e.g. glyphosate tolerant maize monocultures or glyphosate tolerant maize-soybean rotations), little diversity in weed control practices and no mandated herbicide resistance programmes (Waltz et al., 2010), glyphosate resistant weeds may evolve and spread rapidly (e.g. Dauer et al., 2009, Owen et al., 2011). This in turn may induce modification of farmers' weed management practices through intensification of herbicide usage and subsequent adverse environmental effects (Johnson et al., 2009, Kruger et al., 2009b, Shaw et al., 2009, Webster and Sosnovski, 2010). In regions where glyphosate resistant weeds have to be controlled, farmers might exacerbate this phenomenon by increasing rates of glyphosate applied, which may further increase the selection pressure on weeds and lead to more instances of resistance (Duke, 2005, Pline-Srnic, 2005, Neve, 2008, Owen et al., 2011). Populations of five resistant weed species have been found in Europe in 2011 (Spain, Czech Republic, France and Italy) where their occurrence is so far restricted to perennial crops (viniculture, orchards of fruit, citrus or olives) which receive two to four glyphosate applications per year.

While the scale of glyphosate resistant weed outbreaks has remained relatively small so far, a concern is that glyphosate resistant weeds would become more widespread in the near future (Service, 2007), and that this would represent a significant threat to the sustainability of the herbicide and trait, and perhaps to global food production (Duke and Powles, 2008a, Powles, 2010, Owen et al., 2011, Ronald, 2011). Without proper management, the potential for weed populations to express a high degree of resistance to glyphosate will adversely impact the utility of glyphosate (Shaw et al., 2011).

In summary, the cultivation of GMHT crops in monoculture or in rotation with other glyphosate tolerant crops, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides will cause changes in weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. This, in turn, may affect food webs, and the functional value of weed vegetation for organisms of higher trophic levels (reduced functional biodiversity). However, where there is more diversity in weed control practices and crop rotation, and where mandated herbicide resistance programmes are put in place, the selection pressure of glyphosate on weeds will be reduced, decreasing the selection of more tolerant or resistant weeds significantly.

The complex nature of all these dynamic effects will of course be further modulated by market forces and agricultural economics.

Glyphosate can also have effects on soil microbial communities, micorhizal fungi and rhizobial populations important in plant nutrient cycling (Zablotowicz and Reddy, 2004, 2007, Means et al., 2007, Powell et al., 2009a). Zablotowicz and Reddy (2004) reported that glyphosate was toxic to certain *Rhizobia* involved in root nodulation and nitrogen fixation in comparison with herbicides used on conventional soybean. The consequences of this could be that glyphosate applications will reduce rhizobial populations, at least temporarily, thus reducing microbial functions and contributions to field ecosystems – principally in relation to fixing nitrogen. An increased representation of GM non-mycorrhizal hosts within plant communities may indirectly negatively impact beneficial ecosystem services associated with arbuscular mycorrhiza.This could lead to increases in synthetic nitrogen application with consequences for the environment, especially water run-off etc. Powell et al. (2009b) observed that glyphosate use significantly reduces maize litter decomposition although the glyphosate effect is dependant on the location of litter placement.

Thus, whilst it may be easy to list the environmental advantages and disadvantages of the adoption of GMHT systems, it is by no means simple to conclude on overall environmental impacts. Experimentation with GMHT systems can be very expensive, particularly because of the need for farm-scale evaluations on a range of sites with sufficient replication (Perry et al., 2003, Qi et al., 2008) and the need to sample a wide range of biodiversity (Firbank et al., 2003a) over a sufficiently long period (Lutman et al., 2008). When, for whatever reason, experimentation is deemed infeasible, modelling may be attempted, particularly to assess regional-scale and long-term effects of possible changes in agricultural practice over the course of many rotations. However, present models do not provide a robust means of predicting outcomes, because of their critical dependence on underlying assumptions. Different models of the same system may give very different predictions and therefore caution must be exercised in reviewing the output of models. As an illustration, consider four models that were built around the GMHT cropping systems studied in the FSE. In an initial assessment, Heard et al. (2003a,b) used long-term data from the decline in UK weed seedbanks and compounded this with the reduction in seedbank density found for dicotyledons in GMHT crops *other than maize* (e.g. for beet and oilseed rape). They predicted a worst-case decline in seedbanks of 7% per annum for a 5-course cereal rotation with a break crop grown every 5 years. By contrast, they believed that it was quite possible that, under rotations including glufosinate-tolerant maize, weed populations would in the long term be stable or increase. Heard et al. (2005) later revised and refined their earlier opinion for GMHT beet and rape, after taking into account density dependence of the weeds that integrated both population dynamics and grower response to weeds, within a 7-course, 4-year rotational framework. Gibbons et al. (2006) calculated the quantitative effects of changes in seed rain on the dietary requirements of 17 granivorous farmland bird species, although they declined to predict effects on individual bird species. They concluded that should beet, spring and winter rape crops in the UK be largely replaced by GMHT varieties and managed as in the FSE, this would markedly reduce important food resources for farmland birds, many of which had already suffered decline during the last 30 years. By contrast, glufosinate-tolerant maize would be beneficial to farmland birds. Butler et al. (2007) used a semi-qualitative approach and concluded that of 39 susceptible farmland bird species, even under nationwide introduction of the GMHT beet and oilseed rape systems studied in the FSE regimes, only one species would be re-classified to a less favourable conservation status due to the implementation of such systems. Grower uptake was predicted to have only a limited effect on Farmland Bird Indices. Butler et al. (2010) showed that changes in resource availability within the cropped area of agricultural landscapes have been the key driver of current declines in farmland bird populations. The authors developed targeted agri-environment schemes (AES) for cost-effective and efficient delivery of rural development strategies and biodiversity conservation targets. However, it is unclear whether these methodologies will help to manage potential adverse effects of GM HT crops. In conclusion, the potential adverse environmental effects of GMHT crops are (1) the evolution of less desirable weed assemblages leading to a reduction in farmland biodiversity; (2) the evolution of weed resistance and (3) adverse effects on soil microbial communities. The magnitude of these potential adverse environmental effects will depend on the specific herbicide management applied at the farm level. Studies have shown that appropriate management of glyphosate can mitigate some of these potential environmental effects. Dewar et al. (2003), May et al. (2005) Pidgeon et al. (2007) showed that avoiding field margins, row spraying and reduced area spraying in sugar beet could reduce adverse environmental effects without reducing yields.

Management and Monitoring of GMHT crops

The EFSA GMO Panel is of the opinion that an option for the risk management of GMHT crops would be the use of management and mitigation measures (e.g. Dewar et al., 2003, Dewar 2010, May et al., 2005, Pidgeon et al., 2007) to manage herbicide effects on biodiversity, in conjunction with the monitoring for weed resistance evolution under Directive 91/414/EEC (Sweet and Bartsch, 2011). General surveillance (Directive 2001/18/EC) should be used to determine unanticipated adverse environmental effects. As with any other cropping system, the adoption of herbicide tolerant crops will require careful positioning in rotations and monitoring to prevent development of evolved resistance in weeds or a shift in weed spectrum to species with natural higher tolerance (Dewar, 2010). The introduction of GMHT crops has substantially changed weed control systems and sometimes crop rotations and soil tillage systems. Soukup et al. (2011) proposed that attention should be given to the environmental and agro-environmental aspects and sustainability of agro-ecosystems based on HT crops because some adverse effects with this technology can appear, especially if used without knowledge of the risks involved and their prevention. In addition to management measures, they proposed monitoring for potential adverse effects using existing monitoring networks.

Conclusions regarding potential adverse environmental effects of GMHT crops:

- Potential adverse environmental effects are due to the changes in herbicide use and crop management. These include
    - the evolution of less desirable weed assemblages leading to a reduction in farmland biodiversity,
    - the evolution of weed resistance, and
    - negative impacts on soil microbial communities.

- Environmental effects can be managed. The magnitude of these potential adverse environmental effects will depend on the specific herbicide management applied at the farm level in Switzerland as elsewhere.

- Effective management guidelines should be established supported by monitoring to ensure farmer compliance and to assess whether adverse effects are occurring.

4.7.2 Indirect Changes in Susceptibility of HT Crops Against Plant Pathogens

The susceptibility of crops to plant pathogens may theoretically change and result in altered interactions between plant and pathogens (Hilbeck, 2000). Low doses of glyphosate are able to render pathogen-resistant cultivars susceptible to plant diseases (Brammal and Higgins, 1988). On the other hand, this herbicide is also known to be toxic to specific microorganisms, including plant pathogens, and was reported to inhibit or reduce the growth of different plant pathogens, e.g. rust diseases (Feng et al., 2005, Cerdeira and Duke, 2006).

However, increased susceptibility of HT soybean to plant pathogens was reported for Sclerotinia sclerotiorum by Michigan farmers (Lee et al., 2003). Furthermore, an interaction between soybean cyst nematode and glyphosate was also observed (Yang et al., 2002). Colonization of GM soybean roots with fungi was found to be significantly increased after application of glyphosate but not after application of conventional post-emergence herbicides (Sanogo et al., 2001, Njiti et al., 2003, Kremer et al., 2005). Following application of glyphosate to crops, plant root exudates may potentially provide a special advantage to certain Fusarium strains relative to other fungi commonly found in soils (Benbrook, 2005). It is reported that glyphosate in root exudates stimulated growth of selected rhizosphere fungi, possibly by providing a selective C and N source combined with high levels of soluble carbohydrates and amino acids associated with glyphosate treatment of soybean plants. Increased rhizosphere populations of the fungi *Fusarium* spp. and the bacteria Pseudomonas spp. developing under glyphosate treatment of GM soybean may adversely affect plant growth and biological processes in the soil and rhizosphere (Kremer et al., 2005).

In summary, the application of glyphosate in HT crops may potentially affect soil microbial activity and/or soil microbial abundance through exudation of the herbicide into the rhizosphere by HT crops. In addition the use of HT crops has led to more intensive cultivation in short rotations which enhance the development of soil pathogens. As a consequence HT crops may show increased incidence and susceptibility to fungal attacks. These changes could result in increased use of fungicides. As a consequence additional adverse effects on soil microbial activity might occur. However, there are few published results on increased susceptibility of HT crops to fungi and the affects of broad-spectrum herbicides on pathogenic microorganisms.

Conclusions regarding indirect changes in susceptibility of crops against plant pathogens:

- Herbicide treatments and associated changes in rotational management may alter the susceptibility of GMHT crops to plant pathogens.

- Appropriate management and post market environmental monitoring should be applied.

4.7.3 Potential Changes in Fertilizer Use in HT Crops

In glyphosate tolerant soybean, glyphosate is transported from the leaves to the rhizosphere. Glyphosate transported into the roots of HT plants leads to the accumulation of growth inhibiting benzoic acids. It has been reported that application of glyphosate can decrease nodule formation, nodule biomass, nitrogen fixation and nitrogen accumulation (Moorman et al., 1992, King et al., 2001, Powell et al., 2007, Zablotowicz and Reddy, 2007). It has also been reported that symbiotic nitrogen fixation can be affected by herbicides due to direct effects on the rhizobial symbiont as well as due to indirect effects on the physiology of the host plant (Moorman, 1989).

Glyphosate reduces the nitrogenase activity of *Bradyrhizobium japonicum* bacteroids with the inhibition being proportional to the in vitro sensitivity of these strains under culture conditions (Hernandez et al., 1999). The symbiont is known to possess a glyphosate susceptible enolpyryvate-shikimate-synthase (EPSPS) protein (King et al., 2001). Consequently, soybean plants in untreated plots had higher nodule biomass compared to soybean plants in glyphosate-treated plots that were relatively weed-free (Zablotowicz and Reddy, 2007). In addition, a reduction of the N_2 fixation potential could have long-term effects on sustainable soil nitrogen pools (King et al., 2001, Zablotowicz and Reddy, 2004). Soils may lose natural fertility and may lack sufficient nitrogen to produce optimal soybean yields in fields where nitrogen fixation is impaired. This will cause increasing usage of nitrogen fertilizers to maintain the yield levels (Benbrook, 2005).

Another aspect of potential changes in fertilizer use connected with use of the non-selective glyphosate is reported by Eker et al. (2006). In greenhouse experiments the authors sprayed non-glyphosate-tolerant sunflowers (*Helianthus annuus*) with low glyphosate doses (~ 6% of recommended dosage) to simulate effects of glyphosate drifts on non-target plants. In root uptake experiments Fe and Mn uptake were significantly reduced 12 and 24 hours after glyphosate application. This effect may be caused by the formation of poorly soluble glyphosate-metal complexes in plant tissues and or in the rhizosphere as the most important nutrient source since glyphosate is known as an effective chelating agent. Due to the chelating effect poorly soluble glyphosate-metal complexes may be formed in the rhizosphere reducing the availability of these two nutrients.

In summary, the cultivation of HT soybean may affect the nitrogenase activity of symbiotic *Rhizobia* leading to reduced nitrogen fixation. In addition, the chelating effect of glyphosate affects the availability of Mn and Fe for GM crop roots. These fertilizer micronutrients are already recommended to be supplemented in soybean cultivation stewardship programmes depending on soil quality. The effects on soil nutrient mineralization processes of both these glyphosate effects are unclear. However cultivation management of HTGM soya and other plants may result in reduced levels of soil nutrients with adverse effects on nitrogen availability or symbiotic NTOs and uptake of cationic nutrients resulting in potential adverse effects on soil ecological functions. A consequence of this may be that farmers change their fertilizer use and thus have additional environmental impacts. As reviewed by EFSA (2012a) other studies performed under a wide range of environments indicated no yield reductions due to glyphosate applications on glyphosate tolerant soybean, suggesting that soybean has the potential to recover from glyphosate stress (Delannay et al., 1995; Reddy and Whiting, 2000; Elmore et al., 2001; Krausz and Young, 2001; Nelson and Renner, 2001; Reddy and Zablotowicz, 2003). Further, Powell et al. (2009a) reported that nitrogen fixation was greater in GMHT soybean treated with glyphosate than in untreated plants when glyphosate was applied at the first trifoliate soybean growth stage.

Conclusions regarding changes in fertilizer use in GMHT crops:

- GM plant management may cause indirect changes in fertilizer use.
- Appropriate management measures and guidelines including post market environmental monitoring should be applied.

4.7.4 Potential Changes in Landscape Structure

Due to market-orientated and modern production processes the intensity and extent of farming in many regions of the world is currently increasing. On global scale the largest changes in the landscape structure are associated with deforestation to create additional agricultural areas (Begon et al., 2005). Deforestation accounts for 15% of GHG omissions – more than is produced by the entire worlds transport (Clarke, 2012). The rate of deforestation and the soybean acreage (mostly GMHT) nearly doubled from 1995 to 2004 (Trigo and Cap, 2003, Benbrook, 2005, Joensen et al., 2005). In Argentina, the increase in surface area dedicated to GM soybean cultivation has been at the expense of other crops and caused marginalisation of cattle and dairy farming.

In Europe intensification of agricultural production was driven by increased food and feed demands after World War II when the human population started to increase again. The additional rise of production costs led to significant changes in mechanisation to replace labour and changes to the scale of farming units and fields resulting in changes to landscape structure. These changes were often initiated and supported by political programs for supporting agricultural practices (e.g. hedgerow removal, merging of farms and fields) in certain regions. Gardiner et al. (2010) considered that the production of biofuel feedstocks in agricultural landscapes (including the use of GM and non-GM crops) will result in land use changes that may have major implications for arthropod-mediated ecosystem services such as pollination and pest suppression. However, the intensity of landscape changes differs from region to region depending on socio-economic and political factors.

Coexistence measures combined with cultivation of GM crops in Europe may alter farming systems and scales and consequently have impacts on future landscape structure. Farmers who are willing to cultivate GM crops have to fulfill requirements for isolation distances. Fulfillment of these prerequisites may lead to further enlargement or aggregation of cropping fields in order to minimize isolation conflicts to neighbours. This might alter agricultural landscapes by changing field sizes and non cultivated areas such as farm woodlands, hedges, ditches or field margins. These landscape structures are important habitats for maintaining biodiversity in agricultural landscapes (Hendrickx et al., 2007). The connectivity and consequently any potential biological exchange will be negatively affected if these habitats decrease in size and numbers.

According to Collier and Mullins (2011), management impacts can create difficulties when making policy, regulation and licensing decisions in those countries where agriculture has a significant social and ecological position in the landscape. The authors proposed four key biodiversity stressors (Chemicals, Introgression, Nutrients and Management: CINMa) to gauge the potential impacts of the management of a selection of GM crops on an agricultural landscape. An index was developed and applied to five selected GM crops in a case study area: CINMa identified areas in the wider landscape where biodiversity is likely to be negatively or positively impacted, as well as agricultural zones which may benefit from the land use change associated with the management of GM crops and their associated post market environmental monitoring. However, so far there is no experience reported in the literature on the applicability of this index.

No clear impacts on landscapes have been identified in the GM crop production areas of Europe but some changes due to varying national coexistence measures are anticpated with some possible future GM crops.

Conclusions regarding changes in landscape structure:

- GM plant management may cause indirect changes in landscape structure e.g. due to changes in cropping systems and coexistence measures (e.g. larger fields, larger distances between specific crops, changing from livestock to crop production), resulting in loss of habitat connectivity and reduced local biodiversity.

- Appropiate management at the landscape scale combined with post market environmental monitoring should be applied in order to protect biodiversity across landscapes.

5. Risk Evaluation, Mitigation Measures and Environmental Monitoring

5.1 Risk Evaluation

In the EU, the EFSA GMO Panel routinely evaluates existing research and knowledge on biological issues on GM plants of relevance for the European environments.

In addition EU Directive 2001/18/EC (EC 2001) provides the possibility for EU Member States to invoke safeguards on specific GMOs in cases where they consider that new or additional scientific information is likely to affect the risk assessment conclusions on an authorised GMO. Article 34 of Regulation (EC) No 1829/2003, entitled 'Emergency measures', provides that "where it is evident that products authorised by or in accordance with this Regulation are likely to constitute a serious risk to human health, animal health or the environment … measures shall be taken under the procedures provided for in Articles 53 and 54 of Regulation (EC) No 178/2002". Article 53 of Regulation (EC) No 178/2002 provides that "where it is evident that food or feed originating in the Community or imported from a third country is likely to constitute a serious risk to human health, animal health or the environment, and that such risk cannot be contained satisfactorily by means of measures taken by the Member State(s) concerned, the Commission, acting … on its own initiative or at the request of a Member State, shall immediately adopt one or more of the following measures, depending on the gravity of the situation (…)". On 8 September 2011, the EU Court of Justice ruled that "with a view to the adoption of emergency measures, Article 34 of Regulation No 1829/2003 requires Member States to establish, in addition to urgency, the existence of a situation which is likely to constitute a clear and serious risk to human health, animal health or the environment". Furthermore, such measures can be envisaged only if they are supported by a comprehensive risk assessment indicating that such emergency measures are justified.

Such provisions have been invoked by France, Hungary, Greece, and Austria to provisionally prohibit the marketing of several GM plants (e.g. maize MON810, Amflora potato, oilseed rape GT73) for their intended uses in these Member State territories. In the case of MON810 the EFSA GMO Panel examined the set of supporting documents submitted by France, Hungary, Greece, and Austria. In this respect, the GMO Panel assessed whether the submitted documents contain any new scientific information that would change the outcome of previously performed risk assessments, and if detailed grounds exist to consider that the authorised maize MON810, for its intended uses, constitutes a risk to human and animal health or the environment. The GMO Panel looked for evidence for GMO-specific risks taking into consideration the EFSA Guidance Document for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006) as well as any related risk assessments carried out in the past. In addition, the GMO Panel considered the relevance of concerns raised in the light of the most recent scientific data and relevant peer-reviewed publications. After careful evaluation of the submitted documents by these four EU Members, the EFSA GMO Panel concluded that no specific scientific evidence, in terms of risk to human and animal health and the environment, was provided that would justify the invocation of a safeguard clause (EFSA, 2008d,e,f).

The EFSA GMO Panel assessed maize MON810 within a general review of Bt maize and this document provides an extensive overview on the biosafety knowledge on Bt maize with more than 280 literature references (EFSA, 2009a). At the same time the EFSA GMO Panel and the Spanish competent authority have evaluated the renewal application of maize MON810 and this opinion will also contain an extensive review of current information on this maize event and closely related ones.

On 20 February 2012, France notified to the European Commission its scientific argumentation in support of the prohibition of maize MON810 cultivation in the EU, according to Article 34 of Regulation (EC) 1829/2003. The European Commission asked the EFSA GMO Panel to assess if new scientific evidence, that would indicate an environmental concern, was provided by France to support an emergency measure on maize MON810. France endorsed its emergency measure on maize MON810 through a decree[9] of 16 March 2012 suspending the cultivation of maize MON810 varieties.

On 16 April 2012, the European Commission requested the European Food Safety Authority's Panel on Genetically Modified Organisms (EFSA GMO Panel) to assess the supporting documentation submitted by France. In its opinion published 21 May 2012, the EFSA GMO Panel (EFSA, 2012c) considered the relevance of concerns raised by France in the light of the most recent and relevant scientific data published in the scientific literature. In the documentation provided by France in support of the current emergency measure on maize MON810, the EFSA GMO Panel could not identify any new science-based evidence indicating that maize MON810 cultivation in the EU poses a significant and imminent risk to the human and animal health or the environment. The EFSA GMO Panel came to the conclusion is that there is no specific scientific evidence, in terms of risk to human and animal health or the environment, that would support the notification of an emergency measure under Article 34 of Regulation (EC) No 1829/2003 by France and that would invalidate EFSA's previous risk assessments of maize MON810 (EFSA, 2012c).

EFSA (2011a,b) also produced updated opinions on MON810, Bt11 and 1507 maize impacts on non-target lepidoptera and confirmed that low levels of risk can occur when extremely sensitive species are exposed to large scale intensive cultivation of these maizes. EFSA advised that, under such circumstances, measures may need to be taken to reduce exposure levels and hence reduce mortality (see also Section 4.5.1).

In addition the EFSA GMO Panel has produced overall risk assessment opinions on the following GMPs intended for cultivation in the EU:

- glyphosate tolerant maize NK603 and maize GA21 which contain e.g. extensive reviews of information on GM HT crops (EFSA, 2009b, 2011e).
- glyphosate and insect resistant maize MON88017, which contains e.g. an extensive review of information of corn rootworm (*Diabrotica virgifera*) insect resistance management (EFSA, 2011d)

Conclusions regarding risk evaluation:

- Extensive reviews and reports on risk evaluation confirm that new scientific information indicates that the GM plants which have received a positive opinion by EFSA can be regarded as safe as conventional crops for the human and animal health and the environment.
- These reviews also indicate the potential risks associated with the cultivation of HT crops and quantify the low potential risk of cry1Ab and cry 1F producing maize to very sensitive non-target Lepidoptera.

---

[9] http://legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000025525099&categorieLien=id

5.2 Risk Management Including Post Market Environmental Monitoring (PMEM)

The risk assessment can identify risks that require management and propose risk mitigation measures to reduce the levels of risk. In the EU the EFSA GMO Panel provided in their guidelines on the environmental risk assessment of GM plants (EFSA, 2010a) – in line with Annex II of the Directive 2001/18/EC – what scientific requirements should be considered when establishing risk management measures. Risk mitigation should be proportionate to the results of the different risk scenarios studied, the specific protection goals in the receiving environments, and to the levels of scientific uncertainty and risk identified in the environmental risk assessment (EFSA, 2011e)

Directive 2001/18/EC introduces an obligation for applicants to implement monitoring plans, in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans should be designed according to Annex VII of the Directive. The objectives of a post-market environmental monitoring plan according to Annex VII of Directive 2001/18/EC are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct (Case-specific Monitoring); and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the environmental risk assessment (General Surveillance). First experiences with monitoring plans provided by applicants in the EU are reviewed by Bartsch et al. (2006, 2007) and Delos et al. (2007). First experience on the national coordination of GMO monitoring in Germany was published by Gathmann and Bartsch (2006). Allthough it is recommended to agree on common strategies for the data coordination and harmonisation (e.g. Reuter et al., 2011a), there is currently no consensus among competent authorities on important issues e.g. on the baseline against which effects of GMHT cropping must be compared when discussing potential effects on farmland biodiversity (Sanvido et al., 2011b). Another major problem for GS is according to De Jong (2010) that controls are missing with some proposed monitoring methods. Standardization is helpful (see e.g. Seitz et al., 2010) if the methodologies fulfil the necessity and requirements of PMEM according to EFSA (2011e).

The EFSA PMEM guidance (EFSA, 2011e) made use of the experience gained from its assessment of applications on GMPs for cultivation and considered different sources of information such as the PMEM reports on cultivated GMPs, relevant scientific literature and stakeholders comments. EFSA's PMEM guidance (2011e) aims to clarify the objectives, tasks, tools and requirements for PMEM. Firstly, the document explains the scientific rationale for PMEM, including the concept of developing management and monitoring strategies based on the overall conclusions and assumptions of the Environmental Risk Assessment. Secondly, it provides examples and guidance to applicants on how to develop and implement their plans for Case-Specific Monitoring (CSM), taking into account the case-by-case character of CSM. In addition, it provides guidance to applicants on the strategy, methodology and reporting of General Surveillance (GS). Different tools and approaches to implement a plan for GS are considered. EFSA proposes a holistic and integrative approach for monitoring GMPs in the EU that considers GS within a framework of general environmental protection monitoring. Finally, EFSA makes proposals to risk managers for the future conduct of PMEM in the EU and suggests that access to PMEM data could be facilitated by setting-up standardised and centralised reporting centres. In this respect, Smit et al. (2011) described a number of recommendations for the development of a General Surveillance system of the soil ecosystem specifically focussed on the situation in the Netherlands. They concluded that for reasons of cost effectiveness, a GS system of the soil ecosystem will have to make use of existing networks.

5.2.1 Mitigating and Monitoring the Effects of Bt Crops on TOs and NTOs

In the risk assessments of Bt maize and associated EFSA opinions (2005a,b, 2009a, 2011a,b,c,d) it was concluded that resistance to the Bt proteins is likely to evolve in exposed populations of lepidopteran target pest species, particularly those subjected to the highest selection pressures, such as in areas of continuous or very extensive Bt maize cultivation or in rotation with other crops expressing a comparable Bt protein (if any). While this is not considered a direct environmental harm, the consequences of resistance evolution may require altered pest control practices with higher environmental load. Considering that lepidopteran target pests may evolve resistance to Bt-expressing maize under conditions of continuous exposure, the applicants usually propose to put in place risk management measures to delay the possible evolution of resistance. Appropriate IRM strategies (i.e., 'high dose/refuge' strategy) should be employed, in order to delay the potential evolution of resistance to the Bt protein in target pests.

Risk assessments have identified that some extremely sensitive Lepidoptera may be at risk if their larvae are exposed to pollen from certain Cry1 expressing Bt maize. Risks were specifically identified in areas where there are likely to be high levels of exposure through extensive cultivation of Bt maize and where the highly sensitive larvae are feeding on plants when pollen is deposited.

In several opinions EFSA has indicated that monitoring of these Lepidoptera would be problematic due to their generally low level of abundance and the distribution of their host plants and hence larval populations. EFSA therefore suggested that host plant presence in and near Bt maize as well as in the landscape should also be assessed in order to determine exposure levels of host plants and therefore the potential exposure of Lepidoptera populations.

Monitoring should be appropriate to the level of identified risk. For example, an analysis of an existing dataset on butterfly communities in Switzerland (Aviron et al., 2008, 2009) have shown that case-specific monitoring would at best detect large effects in ubiquitous butterfly populations. These authors and Lang (2004) also indicated that monitoring butterfly populations, particularly of infrequent species, is unlikely to achieve the level of sensitivity commensurate with the effects that are anticipated by the EFSA GMO Panel in case of MON810, Bt11 and 1507 maize, unless thousands of samples are taken (EFSA, 2009a). Case-specific monitoring of most lepidotera species would not detect minor shifts in non-target Lepidoptera and therefore needs to be complimented with other studies of the species present in an area and the distribution and frequency of their host plants.

In the case of maize 1507 the EFSA GMO Panel recommended carrying out further field studies on non-target Lepidoptera (see EFSA, 2011c) and considered that the purpose of these studies should be:

- to estimate whether non-target Lepidoptera larvae, with high sensitivity to the Cry1F protein, are in reality feeding on plants in and adjacent to maize fields at the time of pollen deposition, and if so:
  - to estimate the proportions of these populations likely to be affected;
  - to determine the overall effect on maintaining a favourable status of these populations.

The EFSA GMO Panel considered that monitoring and additional studies are only required in situations where there is a potential risk to populations of sensitive non-target Lepidoptera due to high adoption of Bt maize (e.g. in case of maize 1507 an adoption rate of >20%).

The objective of general surveillance is to identify unforeseen adverse effects of the GM plant or its use on human health and the environment that were not predicted in the risk assessment. The general surveillance proposed by applicants in the EU is based on four pillars: (1) the use of annual farm questionnaires to feed a general surveillance database; (2) the review of scientific information provided by existing observation networks; (3) the implementation of company stewardship programs; and (4) the follow-up of various information sources such as official websites, scientific publications and expert reports on GMOs to identify potential adverse effects associated with the intended uses of GM crops like maize MON810 (EFSA, 2009a). General surveillance for the environmental effects of Bt crops cultivation should be in line with the general recommendations of EFSA's guidance on post-market environmental monitoring (EFSA, 2006b, 2011e).

Wilhelm et al. (2010) reviewed PMEM programs of GM Bt maize in view of existing experiences from cultivation, current monitoring activities initiated by Member States and applicants, proposed monitoring strategies and methods as well as potential environmental impacts of cultivation. They concluded that future challenges will arise from large-scale and cumulative cultivation of various GMP events. This will demand optimized organization structures for data collation and integration to support further decision-making and management.

Conclusions regarding monitoring of GM Bt crops:

- Monitoring of resistance evolution in TOs is a general requirement for all Bt crops so far considered by EFSA. This should be associated with appropriate and affective resistance management strategies.
- Monitoring should be practical and feasible in relation to the NTO species potentially at risk from GM Bt crops and appropriate to the level of identified risk.
- General surveillance for the unanticipated environmental effects of GM Bt crop cultivation should be in line with the general recommendations of EFSA.

5.2.2 Monitoring the Effects of Other GMP

Potential adverse environmental effects of the cultivation of GM HT crops are associated with the use of the complementary herbicide regimes (see chapter 4.7.1, and EFSA, 2011e, 2011f). These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, food webs and the ecological functions they provide. The magnitude of these potential adverse environmental effects will depend upon a series of factors, including the specific herbicide and cultivation management applied at the farm level, the crop rotation and the characteristics of receiving environments. Risk mitigation should be proportionate to the results of the different risk scenarios studied, the specific protection goals, the receiving environments, and to the levels of scientific uncertainty and risk identified in the environmental risk assessment (see EFSA, 2011e). In considering the form that case-specific monitoring should take, the EFSA GMO Panel reiterated the considerable challenges it identified previously (EFSA, 2009c, 2011e) to the drawing of meaningful conclusions on the environmental consequences of the use of herbicides from large-scale multi-site experiments, such as the FSEs, which seek to compare HT with conventional herbicide management (Squire et al., 2003, 2009). On the grounds of scientific practicability (e.g. Perry et al., 2003) and of cost (e.g. Qi et al., 2008), and the fact that certain herbicides like glyphosate are already extensively used in a wide range of crops, such studies are considered disproportionate to the identified risks. In order to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and inorder to reduce the remaining scientific uncertainty, the EFSA GMO Panel recommended recently case-specific monitoring to address (1) changes in botanical diversity within fields due to novel herbicide regimes, and (2) resistance evolution to glyphosate in weeds due to novel herbicide regimes (EFSA, 2011f).

Beckie et al. (2010) outlined a framework and protocol for case-specific environmental monitoring of drought-tolerant plants such as canola (*Brassica napus* L.) as a model for GMP with 2nd generation traits. They proposed that the primary potential environmental risk associated with cultivation of drought-tolerant canola is increased invasiveness of volunteers or feral plants (self-perpetuating populations) and weedy relative-crop hybrids or backcrossed progeny in ruderal (noncropped disturbed) and natural areas adjacent to GMP cultivation, resulting in loss of abundance or biodiversity of native plant species. Beckie et al. (2010) stated that accurately predicting invasiveness a priori is problematic, especially for traits that may enhance plant fitness and invasiveness. For them, PMEM can effectively address the greater uncertainties in the environmental risk assessment of these second-generation vs. first-generation GMPs.

Conclusions regarding monitoring of other GM crops:

- Monitoring should be practical and feasible in relation to the ecosystem components potentially at risk from these GM crops and appropriate to the levels of identified risk.
- General surveillance for the unanticipated environmental effects of the GM crop cultivation should be in line with the general recommendations of EFSA.

6. Coexistence Analysis

The EU research project SIGMEA studied coexistence of GM and non-GM crops in Europe and this project included several partners from Switzerland and incorporated many of the coexistence studies conducted in Switzerland in its analysis (Messean et al., 2009).

SIGMEA analysed available information on gene flow in maize, oilseed rape, beet, wheat and rice and established landscape models (Castellazi et al, 2010) and decision support systems to enable coexistence measures, such as crop separation, to be established at field and landscape level. In addition the project studied the economic impacts of proposed coexistence measures (see review by Carpenter, 2010) and legal and liability issues associated with coexistence and the cultivation of GM crops. The studies showed that coexistence was feasible in most situations but would become more problematic with large scale adoption of open pollinating GM crops such as maize (Le Bail et al., 2010, Ruhl et al., 2011) and oilseed rape (Lecomte et al., 2007), especially in areas where fields were small and farms very fragmented.

Many of the studies conducted in SIGMEA were reported in the Third International Conference on Coexistence in Seville in 2007 (GMCC07) published by the European Commission (ISBN 978-92-79-07298-7) and available at http://ipts.jrc.ec.europa.eu.

SIGMEA has recently produced a summary report of its main findings and this is attached as Annex. One of the main conclusions of SIGMEA is that coexistence measures should be adopted on a case by case basis taking into account the nature of the crop, the relative areas of GM and non-GM crops, the juxtaposition of other crops, fields and farms, landscape and climatic features. Establishing standard isolation measures for each crop would require setting isolation distances that would achieve the desired thresholds under most circumstances. In many cases this would result in disproportionate measures being adopted with associated costs and management issues. More information on SIGMEA is available at www.inra.fr/sigmea. The EU research project Co-Extra has also studied coexistence and traceability from farm to fork, (e.g. right through production, processing and supply chains). This project adds information on stacked genes on agricultural coexistence (Paul et al, 2011), and provides considerable insight into methods for restricting gene flow including cleistogamy in oilseed rape, cytoplasmic male sterility (Weider et al., 2009), refugia and transplastomics.

Of particular interest was the study on the use of cytoplasmic male sterility and xenia in maize for the biological containment of transgenes while maintaining high levels of crop productivity. This study was conducted partly within NFP59 and also within Coextra and SIGMEA. It was lead by Peter Stamp's group at ETH and demonstrated that the Plus hybrid system, i.e. growing suitable mixtures of GM cytoplasmic male-sterile plants (80%) and unrelated non-GM male fertile plants (20%), the latter acting as pollen donors, is an interesting way for controlling the release of pollen from genetically modified maize (Weider et al., 2009). The Plus-hybrid system relies on the fact that the female fertility of CMS plants is not affected and seeds can be set if vital pollen is provided. Field trials were conducted in several European countries over a 3 year period.

Coextra also studied economic and logistical aspects of supply chain management and provided considerable information on sampling techniques (Sustar-Vozlic et al., 2011) and new diagnostic techniques (Gašparič, et al., 2010; Papazova et al., 2010, Bahrdt et al., 2010), including those for unknown GM admixtures (Ruttink et al, 2011) and stacked genes (Paul et al, 2011). Information is available at www.coextra.eu.

Swiss researchers from AGRO Reckenholtz (Sanvido et al., 2008b) have reviewed existing cross-fertilization studies in maize, established relevant criteria for the evaluation of these studies and applied these criteria to define science-based isolation distances. To keep GM-inputs in the final product well below the 0.9% threshold defined by the EU, isolation distances of 20 m for silage and 50 m for grain maize, respectively, are proposed based on data from several studies as recently reviewed by Riesgo et al. (2010). An evaluation using statistical data on maize acreage and an aerial photographs assessment of a typical agricultural landscape by means of Geographic Information Systems (GIS) showed that spatial resources would allow applying the defined isolation distances for the cultivation of GM maize in the majority of the cases under actual Swiss agricultural conditions. A different view is expressed by researchers from FIBL Frick (Oehen et al., 2007, Oehen and Stolze, 2009) who investigated the coexistence costs of GM and Non-GM grain maize for the GM maize and the Non-GM maize producer based on isolation distances of 100 m. From their simulation results the authors concluded that the benefits of GM maize production can only cover the potential coexistence and management costs in cases of i) small isolation zone distances and ii) high GM maize adoption rates of 90%.

Gene flow and coexistence studies have also been conducted in N and S America, Australia and some other countries (Stein and Rodrígez-Cerezo, 2010; Kaphengst et al., 2010). Results are largely similar to those in Europe but other crops have also been studied in some of these countries including grasses, alfalfa (lucern), and sunflower. These results have been recently summarised in a conference held in Washington in September 2011 (see Annex). The Coexistence Bureau for the European Union has published a best practice document for the coexistence of maize (Rodrígez-Cerezo and Czarnak-Kłos 2010) and other docucuments will follow.

Conclusions regarding coexistence and segregation:

- The studies showed that coexistence is feasible in most situations, but depends on isolation measures and segregation costs.
- Coexistence would become more problematic with large scale adoption of open pollinating GM crops such as maize and oilseed rape, especially in areas where fields were small and farms very fragmented.
- Coexistence is more problematic in areas that include both organic and GM open pollinating crops as organic growers are often required to have “zero” admixture with GM crops in their produce (Stokstad, 2011), even though there is no legal basis for a lower threshold in most European countries.
- Segregation of processing and supply chains would require the introduction of new measures in many systems and also involve additional costs.
- Cultivation of GM maize in Europe has not raised major problems for coexistence as much of the maize is fed to livestock and so there is no necessity for segregation pre and post harvest in most countries. Arrangements with organic growers have included manipulation of sowing dates and isolation in order to avoid pollination of organic crops.
- Cultivation of GM potatoes for starch is being done in a closed system which involves complete separation of the cultivation, harvest and processing from other potatoes. Some processed materials are used in biogas production and some will be fed to livestock in future but no segregation problems are predicted.

7. General Conclusions for ERA and Coexistence

In more than 20 years of experimental field releases and more than 10 years of commercial cultivation, adverse effects reported in the scientific literature concern (i) adverse effects on non-target organisms (ii) the development of resistance in Bt crop target organisms and (iii) reduced biodiversity and increased weed tolerance caused by complementary herbicides used in HT crops. No other adverse environmental effects of approved crops have been reported in the scientific literature though there have been several unsubstantiated reports of adverse effects (e.g. adverse effects on animal or human health). Impacts on non-target organisms have been shown in (some) laboratory studies but field studies indicate that effects on populations are within the normal range found in agricultural systems. An important lesson is that negative effects observed in the laboratory do not necessarily predict impacts in the field, where many other factors can affect impacts on non target species (including climate, food availability and predation). Resistance development in plant pests targeted by GM crops expressing protective Bt proteins, tolerance in weeds and reduced biodiversity caused by complementary herbicides used on HT crops, are effects which were already anticipated from the risk assessment studies and the scientific literature.

However, other potential effects are discussed in the relevant chapters above and in the scientific literature.

Gene flow regarding GM traits from GM crop plants to wild relatives should be considered in cases of GM plants that have ancestors in the natural European flora, e.g. for crops related to vegetable, tree fruit and herbage species which have sexually compatible relatives. Although gene flow as such is not an adverse environmental effect, the long-term consequences for species fitness, conservation and biodiversity might be relevant.

Coexistence between different types of crops is an important agro-economic issue which has to be addressed once GM crops are approved. Risk management and coexistence measures should be considered together. Thus non-GM buffer zones around Bt crops to restrict cross pollination can also be used as refugia in pest resistance management.

The art of Environmental Risk Assessment is constantly evolving and incorporating new scientific information and experiences. In addition the information being asked of risk assessors is becoming more complex and demanding. Long term, cumulative effects are difficult to determine against a background of flux in biota, ecosytems, agriculture, environments and global climate change. The introduction of complex GM plants containing a number of events will increase the data requirements for risk assessment and increase the complexity of the analyses and assessments.

8. Summary of Practical Consequences for Switzerland

This report shows that there are potentially several GM crops of interest to Switzerland currently or shortly to be available. It also shows that the identified environmental risks associated with GM crops lie within the range of risks currently found in conventional crop production. For example the environmental and agronomic impacts of cultivating Bt and HT crops are similar to those associated with the crop protection measures currently applied to conventional crops. However it is recognised that, as well as the range of scientific, environmental and agronomic factors affecting their introduction, there will also be a range of socio-economic factors to consider. Switzerland will need to decide what its future strategy is for developing sustainable agriculture and protecting agricultural environments. This strategy will determine the environmental goals that are required and influence the role that GM crops can play in achieving these goals.

9. Literature

Abbott, R.J., James, J.K., Milne, R.I., Gillies, A.C.M. (2003): Plant introductions, hybridization and gene flow. Philosophical Transactions of the Royal Society, B 358: 1123-1132.

Abou-Elwafa, S.F., Büttner, B., Kopisch-Obuch, F.J., Jung, C. Müller, A. (2012): Genetic identification of a novel bolting locus in Beta vulgaris which promotes annuality independently of the bolting gene B. Molecular Breeding, 29, p. 989–998.

Abt, E., Rodricks, J.V., Levy, J.I., Zeise, L., Burke, T. (2010): Science and Decisions: Advancing Risk Assessment. Risk Analysis 30, 1028-1036. DOI:10.1111/j.1539-6924.2010.1426.x.

ACRE (2007a): Minutes of the 116th meeting of ACRE at the University of Essex, Colchester, Thursday 6th December 2007, Point 9 Research paper: toxins in trangene crop byproducts may affect headwater stream ecosystems, ACRE/07/P31.
http://www.defra.gov.uk/environment/acre/meetings/07/min071206.htm

ACRE (2007 b): Managing the footprint of agriculture: towards a comparative assessment of risks and benefits for novel agricultural systems. DEFRA, London.
http://www.defra.gov.uk/environment/acre/fsewiderissues/pdf/acre-wi-final.pdf

AGBIOS (2009): http://www.agbios.com/dbase.php (accessed 9 May, 2009).

Ahmad, A., Wilde, G.E., Zhu, K.Y. (2005): Detectability of coleopteranspecific Cry3Bb1 protein in soil and its effect on nontarget surface and below-ground arthropods. Environmental Entomology 34: 385-394.

Ahmad, A., Wilde, G.E, Withworth, R.J., Zolnerowich, G. (2006a): Effects of corn hybrids expressing the coleopteran-specific Cry3Bb1 protein for corn rootworm control on aboveground insect predators. Journal of Economic Entomology 99: 1085-1095.

Ahmad, A., Wilde, G.E., Zhu, K.Y. (2006b): Evaluation of effects of coleopteran-specific Cry3Bb1 protein on earthworms exposed to soil containing corn roots or biomass. Environmental Entomology 35: 976-985.

Ahmad, R., Kim, M.D., Back, K.H., Kim, H.S., Lee, H.S., Kwon, S.Y., Murata, N., Chung, W.I., Kwak, S.S. (2008): Stress-induced expression of choline oxidase in potato plant chloroplasts confers enhanced tolerance to oxidative, salt, and drought stresses. Plant Cell Reports 27(4):687-98. DOI:10.1007/s00299-007-0479-4.

Akhtar, Z.R., Tian, J.C., Chen, Y., Fang, Q., Hu, C., Chen, M., Peng, Y.F., Ye, G.Y. (2010): Impacts of Six Bt Rice Lines on Nontarget Rice Feeding Thrips Under Laboratory and Field Conditions. Environmental Entomology 39 (2), 715-726. DOI:10.1603(EN09095).

Al Mouemar, A., Darmency, H. (2004): Lack of stable inheritance of introgressed transgene from oilseed rape in wild radish. Environmental Biosafety Research 3: 209-214.

Albajes, R., Eizaguirre, M., Casado, D., Pérez, M., López, C., Lumbierres, B., Pons, X. (2007): Impact of glyphosate use on arthropods related to the cultivation of transgenic herbicide-tolerant maize. IOBC/wprs Working Group 'GMOs in Integrated Plant Production', 3rd EIGMO Meeting "Ecological Impact of Genetically Modified Organisms (EIGMO)".
http://iobc-gmo.sggw.pl/image/programe.pdf

Albajes, R., Lumbierres, B., Pons, X. (2009): Responsiveness of arthropod herbivores and their natural enemies to modified weed management in corn. Environ Entomol 38(3):944-954.

Albajes, R., Lumbierres, B., Pons, X. (2010): Managing weeds in herbicide-tolerant GM maize for biological control enhancement. In: Romeis J (Ed), GMOs in Integrated Plant Production, IOBC wprs Bulletin 52, 1-8.

Albajes, R., Lumbierres, B., Pons, X. (2011): Two heteropteran predators in relation to weed management in herbicide-tolerant corn. Biological Control. DOI:10.1016/j.biocontrol.2011.03.008.

Al-Deeb, M.A., Wilde, G.E., Blair, J.M., Todd, T.C. (2003): Effect of Bt corn for corn rootworm control on nontarget soil microarthropods and nematodes. Environmental Entomology 32, 859-865.

Allainguillaume, J., Alexander, M., Bullock, J.M., Saunders, M., Allender, C.J., King, G., Ford, C.S., Wilkinson, M.J. (2006): Fitness of hybrids between rapeseed (*Brassica napus*) and wild *Brassica rapa* in natural habitats. Molecular Ecology 15: 1175-1184. DOI:10.1111/j.1365-294X.2006.02856.x.

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., Handelsman, J. (2010): Call of the wild: antibiotic resistance genes in natural environments. Nature Reviews Microbiology: 1-9. DOI:10.1038/nrmicro2312.

Alstad, D.A. and Andow, D.A. (1995): Managing the evolution of insect resistance to transgenic plants. Science, 268: 1894-1896.

Alvarez, F., Ortego, F., Castañera, P. (2005): Impact of Bt-maize on the poliphagous predator Poecilus cupreus L. (Coleoptera: Carabidae). IOBC meeting on ecological impacts on GMOs. Lleida, Spain, June 1-3 2005. Proceedings of the IOBC meeting on ecological impacts on GMOs.

Alvarez-Alfageme, F., Bigler, F., Romeis, J. (2010): Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvae of *Adalia bipunctata* (Coleoptera: Coccinellidae): the importance of study design. Transgenic Research. DOI:10.1007/s11248-010-9430-5.

Alvarez-Alfageme, F., Ferry N., Castañera, P., Ortego, F., Gatehouse, A.M.R. (2008): Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator Stethorus punctillum Weise (Coleoptera: Coccinellidae). Transgenic Research, 17: 943-954.

Alvarez-Alfageme, F., Ortego, F., Castañera, P. (2009): Bt maize fed-prey mediated effect on fitness and digestive physiology of the ground predator Poecilus cupreus (Coleoptera: Carabidae). Journal of Insect Physiology, 55: 144-150.

Alves, A.P., Spencer, T.A., Tabashnik, B.E., Siegfried, B.D. (2006): Inheritance of resistance to the Cry1Ab *Bacillus thuringiensis* toxin in *Ostrinia nubilalis* (Lepidoptera: Crambidae). Journal of Economic Entomology 99: 494-501.

Ammitzbøll, H.M, Jørgensen, B. (2006): Hybridization between oilseed rape (*Brassica napus*) and different populations and species of *Raphanus*. Environmental Biosafety Research 5: 3-13. doi10.1051/ebr:2006010.

Andersen, N.S., Siegismund, H.R., Meyer V., Jørgensen, B. (2005): Low level of gene flow from cultivated beets (*Beta vulgaris* L. Spp. vulgaris) in Danish populations of sea beet (*Beta vulgaris* L. spp. *maritima* (L.) Arcangeli). Molecular Ecology (DOI: 10.1111/j.1365-294X.2005.02490.x).

Andersen, M.N., Sausse, C., Lacroix, B., Caul, S., Messéan, A. (2007): Agricultural studies of GM maize and the field experimental infrastructure of ECOGEN. Pedobiologia 51: 175-184. DOI:10.1016/j.pedobi.2007.03.005.

Anderson, K. (2010): Globalization’s effects on world agricultural trade, 1960-2050. Philosophical Transaction of the Royal Society 365, 3007-3021. DOI:10.1098/rstb.2010.0131.

Anderson, P.L., Hellmich R.L., Sumerford, D.V., Lewis, L.C. (2004): Effects of Cry1Ab-expressing corn anthers on monarch butterfly larvae. Environ. Entomol. 33: 1109-1115.

Anderson, P.L., Hellmich, R.L., Prasifka, J.R., Lewis, L.C. (2005): Effects on fitness and behavior or monarch butterfly larvae exposed to a combination of Cry1Ab-expressing corn anthers and pollen. Environ. Entomol. 34: 944-952.

Andow, D.A. (2003): UK farm-scale evaluations of transgenic herbicide-tolerant crops. Nature Biotechnology 2: 1453-1454.

Andow, D.A. (2008): The risk of resistance evolution in insects to transgenic insecticidal crops. Collect. Biosafety Rev. 4: 142-199.

Andow, D.A. and Alstad, D.A. (1998): F2 screen for rare resistance alleles. J. Econ. Entomol. 91: 572-578.

Andow, D.A., Birch, A.N.E., Dusi, A.N.E., Fontes, M.G., Hilbeck, A., Lang, A., Lövei, G.L., Pires, C.S.S., Sujii, E.R., Underwood E., Wheatley, R.E. (2006b): Non-target and biodiversity risk assessment for genetically modified (GM) crops. Proceedings of the Ninth International Symposium on the Biosafety of Genetically Modified Organisms, Korea, September 2006: 68-73.

Andow, D.A., Farrell, S.L., Hu, Y. (2010): Planting patterns of in-field refuges observed for Bt maize in Minnesota. Journal of Economic Entomology 103 (4), 1394-1399. DOI:10.1603/EC09201.

Andow, D.A. and Hilbeck, A. (2004): Science-based risk assessment for nontarget effects of transgenic crops. BioScience 54: 637-649.

Andow, D.A., Lövei, G.L., Arpaia, S. (2006a): Ecological risk assessment for Bt crops. Nat. Biotechnol. 24, 749-751.

Andow, D.A., Olson, D.M., Hellmich, R.L., Alstad, D.N., Hutchison, W.D. (2000): Frequency of resistance alleles to *Bacillus thuringiensis* toxin in an Iowa population of European corn borer. J. Econ. Entomol. 93: 26-30.

Andow, D.A., Zwahlen, C. (2006): Assessing environmental risks of transgenic plants. Ecology letters 9: 196-214. DOI:10.1111/j.1461-0248.2005.00846.x.

Andreadis, S.S., Alvarez-Alfageme, F.A., Sánchez-Ramos, I., Stodola, T.J., Andow, D.A., Milonas, P.G., Savopoulou-Soultani, M., Castánera, P. (2007): Frequency of resistance to *Bacillus thuringiensis* toxin Cry1Ab in Greek and Spanish population of *Sesamia nonagrioides* (Lepidoptera: Noctuidae). J. Econ. Entomol. 100: 195-201.

Anonymous (2006): Monitoring of the environmental effects of the Bt gene. Schriftenreihe Bayerische Landesanstalt Landwirtschaft 10/2006, http://www.lfl-neu.bayern.de/publikationen/daten/schriftenreihe_url_1_43.pdf (accessed 21 January, 2009).

Aono, M., Wakiyama, S., Nagatsu, M., Nakajima, N., Tamaoki, M., Kubo, A., Saji, H. (2006): Detection of feral transgenic oilseed rape with multiple-herbicide resistance in Japan. Environmental Biosafety Research 5: 77-87. DOI:10.1051/ebr:2006017.

APHIS (2005): Approval of Monsanto Company request 04-125-01p seeking a determination of non-regulated status for corn rootworm resistant MON 88017, http://www.aphis.usda.gov/brs/aphisdocs2/04_12501p_com.pdf

Arber, W. (2010): Genetic engineering compared to natural genetic variations. New Biology 27 (5).

Arnaud, J.F., Fenart, S., Cordellier, M., Cuguen, J. (2010): Populations of weedy crop-wild hybrid beets show contrasting variation in mating system and population genetic structure. Evolutionary Applications, 305-318. DOI:10.1111/j. 1752-4571.2010.00121.x.

Arnold, M.L. and Hodges, S.A. (1995): Are natural hybrids fit or unfit relative to their parents? Trends in Ecology and Evolution 10: 67-71.

Arpaia, S. (2010): Genetically Modified Plants and “Non-Target” Organisms: Analysing the Functioning of the Agro-ecosystem. ICGEB review paper.

Arregui, M.C., Sanchez, D., Althaus, R., Scotta, R.R., Bertolaccini, I. (2010): Assessing the risk of pesticide environmental impact in several Argentinian cropping systems with a fuzzy expert indicator. Pest Management Science 66 (7), 736-740. DOI:10.1002/ps.1935.

Arrigo, N., Guadagnuolo, R., Lappe, S., Pasche, S., Parisod, C., Felber, F. (2011): Gene flow between wheat and wild relatives: empirical evidence from *Aegilops geniculata*, *Ae. neglecta* and *Ae. triuncialis* Evolutionary Applications 4, p. 685-695.

Ashouri, A., Michaud, D., Cloutier, C. (2001): Unexpected effects of different potato resistance factors to the Colorado potato beetle (Coleoptera: Chrysomelidae) on the potato aphid (Homoptera: Aphididae). Environmental Entomology 30: 524-535.

Aviron, S., Sanvido, O., Herzog, F., Baudry, J., Romeis, J., Bigler, F. (2006): Monitoring effects of GM crops on butterflies: the use of multiscale approaches for general surveillance. J. Consum. Protec. Food Safety *1(S1):* 85-88.

Aviron, S., Sanvido, O., Romeis, J., Herzog, F., Bigler, F. (2009): Case-specific monitoring of butterflies to determine potential effects of transgenic Bt-maize in Switzerland. Agriculture, Ecosystems and Environment, 131: 137-144.

Babendreier, D., Kalberer, N.M., Romeis, J., Fluri, P., Bigler, F. (2004): Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. Apidol. 35: 293-300.

Babendreier, D., Kalberer, N.M., Romeis, J., Fluri, P., Mulligan, E., Bigler, F. (2005): Influence of Bt-transgenic pollen; Bt-toxin and protease inhibitor (SBTI) ingestion on development of the hypopharyngeal glands in honeybees. Apidologie 36: 585-594. DOI:10.1051/apido:2005049.

Bagavathiannan, M.V., Gulden, R.H., Begg, G.S., Acker, R.C. van (2011b): The demography of feral alfalfa (Medicago sativa L.) populations occurring in roadside habitats in Southern Manitoba, Canada:

implications for novel trait con-finement. Environmental Science and Pollution Research 17, 1448-1459.

Bagavathiannan, M.V., Spok, A., Acker, R. C. van (2011a): Commercialization of perennial GE crops: looming challenges for regulatory frameworks.Journal of Agricultural & Environmental Ethics 24, 227-242.

Bagla, P. (2010): Hardy cotton-munching pests are latest blow to GM crops. Science 327, p. 1439.

Bahrdt, C., Krech, A.B., Wurz, A., Wulff, D. (2010): Validation of a newly developed hexaplex real-time PCR assay for screening for presence of GMOs in food, feed and seed. Analytical and Bioanalytical Chemistry 396, 2103-2112. DOI:10.1007/s00216-009-3380-x.

Bai, Y.Y., Jiang, M.X., Cheng, J.A. (2005): Effects of transgenic cry1Ab rice pollen on fitness of Propylea japonica (Thunberg Journal of Pesticide Science 78: 123-128. DOI:10.1007/s10340-004-0078-x.

Bai, Y.Y., Yan, R.H., Ye, G.Y., Huang, F.N., Cheng, J.A. (2010): Effects of Transgenic Rice Expressing *Bacillus thuringiensis* Cry1Ab Protein on Ground-Dwelling Collembolan Community in Postharvest Seasons. Environmental Entomology 39 (1), 243-251. DOI:10.1603/EN09149.

Bailey, R.I., Bourguet, D., Le Pallec, A.H., Ponsard, S. (2007): Dispersal propensity and settling preferences of European corn borers in maize field borders. J. Appl. Ecol. 44: 385-394.

Bailey, J., Scott-Dupree, C., Harris, R., Tolman, J., Harris, B. (2005): Contact and oral toxicity to honey bees (*Apis mellifera*) of agents registered for use for sweet corn insect control in Ontario, Canada. Apidologie 36: 623-633.

Bakonyi, G., Dolezsai, A., Mátrai, N., Székács, A. (2011): Effects of Consumption of Bt-maize (*MON 810*) on the Collembolan *Folsomia candida*, Over Multiple Generations: A Laboratory Study. Insects 2, 243-252. DOI:10.3390/insects2020243.

Bakonyi, G., Szira, F., Kiss, I., Villányi, I., Seres, A., Székacs, A. (2006): Preference tests with collembolas on isogenic and Bt-maize. European Journal of Soil Biology: 132-135. DOI:10.1016/j.ejsobi.2006.06.005.

Balog, A., Kiss, J., Szekeres, D., Szénási, Á., Markó, V. (2010): *Rove beetle* (Coleoptera: Staphylinidae) communities in transgenic *Bt* (MON810) and near isogenic maize. Crop Protection: 1-5. DOI:10.1016/j.cropro.2009.12.020.

Balog, A., Szénási, A., Szekeres, D., Pálinkás, Z. (2011): Analysis of soil dwelling rove beetles (Coleoptera: Staphylinidae) in cultivated maize fields containing the Bt toxins, Cry34/35Ab1 and Cry1F x Cry34/35Ab1. Biocontrol Science and Technology, 1-5. DOI:10.1080/09583157.2010.545104.

Baltazar, B.M., de Jesús Sánchez-Gonzalez, J., de la Cruz-Larios, Schoper, J.B. (2005): Pollination between maize and teosinte: an important determinant of gene flow in Mexico. Theor. Appl. Genet. 110: 519-526.

Barros, E., Lezar, S., Anttonen, M.J., van Dijk, J.P., Röhling, R.M., Kok, E.J., Engel, K.-H. (2010): Comparison of two GM maize varieties with a near-isogenic non-GM variety using transcriptomics, proteomics and metabolomics. Plant Biotechnology Journal 8, 1-16. DOI:10.1111/j. 1467-7652. 2009.00487.x.

Bartsch, D. (2011): Gene Flow in Sugar Beet. Sugar Tech. DOI:10.1007/s12355-010-0053-1.

Bartsch, D., Bigler, F., Castanera, P., Gathmann, A., Gielkens, M., Hartley, S., Lheureux, K., Renckens, S., Schiemann, J., Sweet, J., Wilhelm, R. (2006): Concepts for General Surveillance of Genetically Modified (GM) Plants: The EFSA position. Journal für Verbraucherschutz und Lebensmittelsicherheit (Journal of Consumer Protection and Food Safety), Volume 1, Supplement 1, p. 15-20.

Bartsch, D., Cuguen, J., Biancardi, E., Sweet, J. (2003): Environmental implications of gene flow from sugar beet to wild beet – current status and future research needs. Environmental Biosafety Research 2, 105-115.

Bartsch, D., Devos, Y., Hails, R., Kiss, J., Krogh, P.H., Mestdagh, S., Nuti , M., Sessitsch, A., Sweet, J., Gathmann, A. (2010): Environmental Impact of Genetically Modified Maize Expressing Cry1 Proteins. Genetic Modification of Plants, Biotechnology in Agriculture and Forestry 64. DOI:10.1007/978-3-642-02391-0_27.

Bartsch, D., Gathmann, A., Hartley, S., Hendriksen, N.B., Hails, R., Lheureux, K., Kiss, J., Mesdagh, S., Neemann, G., Perry, J. Renckens, S., Schiemann, J., Sweet, J.(2007): First EFSA experiences with monitoring plans. Journal für Verbraucherschutz und Lebensmittelsicherheit (Journal of Consumer Protection and Food Safety), Volume 2, Supplement 1, 33-36.

Bartsch, D., Schmidt, M., Pohl-Orf, M., Haag, C., Schuphan, I. (1996): Competitiveness of transgenic sugar beet resistant to beet necrotic yellow vein virus and potential impact on wild beet populations. Molecular Ecology 5: 199-205.

Bartsch, D. and Schuphan, I. (2002): Lessons we can learn from ecological biosafety research. Journal of Biotechnology 98, 71-77.

Bartsch, D., Wehres, U., Göddecke, U., Gathmann A. (2003): Introduction to field trial data of crop to weed beet gene flow. In: B. Boelt (eds.) Proceedings of GMCC-03 – GM Crops and Co-existence, 13th to 14th November 2003, Danish Institute of Agricultural Sciences, Slagelse, 105-107.

Bartz, R., Heink, U., Kowarik, I. (2009): Proposed Definition of Environmental Damage Illustrated by the Cases of Genetically Modified Crops and Invasive Species. Conservation Biology.

Bates, S.L., Zhao, J.Z., Roush, R.T., Shelton, A.M. (2005): Insect resistance management in GM crops: past, present and future. Nature Biotechnology 23: 57-62. DOI:10.1038/nbt1056.

BATS (1996): Gentechnisch veränderte krankheits- und schädlingsresistente Nutzpflanzen – Eine Option für die Landwirtschaft? Band 2. Zentrum für Biosicherheit und Nachhaltigkeit. http://www.bats.ch/bats/publikationen/gentech-nutzpflanzen/band2-resistenteNutzpflanzen.php?lang_select=en

BATS (2000): Ergebnisse zum Forschungsprojekt Nachhaltige Landwirtschaft und grüne Gentechnik. 149 pages.
http://www.bats.ch/bats/publikationen/nachhaltige_landwirtschaft/nachhaltige_landwirtschaft.pdf

Baumgarte, S. and Tebbe, Ch. (2005): Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Molecular Ecology 14: 2539-2551. DOI:10.1111/j.1365-294X.2005.02592.x.

Beachy, R.N., Fedoroff, N.V., Goldberg, R.B., McHughen, A. (2008): The burden of proof: A response to Rosi-Marshall et al., Proc. Natl. Acad. Sci. USA 105: E9.

Beckie, H.J. (2011): Herbicide-resistant weed management: focus on glyphosate. Pest Management Science, DOI:10.1002/ps.2195.

Beckie, H.J., Hall, L.M., Simard, M-J., Leeson, J.Y., Willenborg, C.J. (2010): A Framework for Postrelease Environmental Monitoring of Second-generation Crops with Novel Traits. Crop Science. DOI: 10.2135/cropsci2009.12.0743.

Beckie, H.J., Harker, K.N., Hall, L.M., Warwick, S.I., Légère, A., Sikkema, P.H., Clayton, G.W., Thomas, A.G., Leeson, J.Y., Séguin-Swartz, G., Simard, M.-J. (2006): A decade of herbicide-resistant crops in Canada. Canadian Journal of Plant Science, 86: 1243-1264.

Beckie, H.J., Séguin-Swartz, G., Nair, H., Warwick, S.I., Johnson, E. (2004): Multiple herbicide-resistant canola (*Brassica napus*) can be controlled by alternative herbicides. Weed Science, 52: 152-157.

Beddington, J. (2010): Global food and farming futures. Philosophical Transaction of the Royal Society 365, 2767. DOI:10.1098/rstb.2010.0181.

BEETLE (2009): Long-term effects of genetically modified (GM) crops on health and the environment (including biodiversity): prioritization of potential risks and delimitation of uncertainties. German Federal Office of Consumer Protection and Food Safety, BLaU-Umweltstudien and Genius GmbH, http://ec.europa.eu/environment/biotechnology/pdf/beetle_report.pdf

Begon, M., Townsend, C., Harper, J.L. (2005): Ecology. From individuals to ecosystems (4th edition). Blackwell Publishing.

Benbrook, C. (2005): Rust, resistance, run down soils, and rising costs – problems facing soybean producers in Argentina. AgBiotechNet 8.

Ben Taher, S., Salva, I., Brants, O.I. (2010): Genetic stability in two commercialized transgenic lines (MON810): Nature Biotechnology 28 (8), 779.

Bennett, P.M., Livesey, C.T., Nathwani, D., Reeves, D.S., Saunders, J.R. (2004): An assessment of the risks associated with the use of antibiotic resistance genes in genetically modified plants: report of the Working Party of the British Society for Antimicrobial Chemotherapy. Journal of Antimicrobial Chemotherapy 53: 418-431.

Berge, J.B., Ricroch, A.E. (2010): Emergence of minor pests becoming major pests in GE cotton in China. GM Crops.

Bergelson, J. (1994): Changes in fecundity do not predict invasiveness: a model study of transgenic plants. Ecology 75: 249-252. DOI:10.2307/1939399.

Bern L, (1990): Postcapture particle size selection by Daphnia cucullata (Cladocera). Limnology and Oceanography 35, 923-926.

Bhatti, M.A., Duan, J., Head, G.P., Jiang, C., McKee, M.J., Nickson, T.E., Pilcher, C.L., Pilcher, C.D. (2005): Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected Bt corn on foliage-dwelling arthropds. Environmental Entomology 34: 1336-1345.

Bigler, F., Albajes, R. (2011): Indirect effects of genetically modified herbicide tolerant crops on biodiversity and ecosystem services: the biological control example. Journal für Verbraucherschutz und Lebensmittelsicherheit. DOI:10.1007/s00003-011-0688-1.

Binning, R.R., Lefko, S.A., Millsap, A.Y., Thompson, S.D., Nowatzki, T.M. (2010): Estimating western corn rootworm (Coleoptera: Chrysomelidae) larval susceptibility to event DAS-59122-7 maize. Journal of Applied Entomology, 1-11. DOI:10.1111/j.1439-0418.2010.01530.x.

Birch, A.N.E., Geoghegan, I.E., Majerus, M.E.N., NcNicol, J.W., Hackett, C.A., Gatehouse, A.M.R., Gatehaouse, J.A. (1999): Tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance. Molecular Breeding 5: 75-83.

Birch, A.N.E., Griffiths, B.S., Caul, S., Thompson, J., Heckmann, L.H., Krogh, P.H. (2007): The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: A review on the ECOGEN project. Pedobiologia 51: 251-260. DOI:10.1016/j.pedobi.2007.04.008.

Bitocchi, E., Nanni, L., Rossi, M., Rau, D., Giardini, A., Bellucci, E., Buonamici, A., Vendramin, G.G., Papa, R. (2009): Introgression from modern hybrid varieties into landrace populations of maize (*Zea mays* ssp. *mays* L.) in central Italy. Mol. Ecol. 18(4): 603-21.

Bitzer, R.J., Rice, M.E., Pilcher, C.D., Pilcher, C.L., Lam, W.K.F. (2005): Biodiversity and community structure of epedaphic and euedaphic springtails (Collembola) in transgenic rootworm Bt corn. Environmental Entomology 34: 1346-1376.

Blackwood, C.B., Buyer, J.S. (2004): Soil microbial communities associated with Bt and non-Bt corn in three soils. Journal of Environmental Quality 33: 832-836.

Blanco, C.A, Gould, F., Groot, A.T., Abel, C.A., Hernandez, G., Perera, O.P., Teran-Vargas, A.P. (2010): Offspring From Sequential Matings Between *Bacillus thuringiensis*-Resistant and *Bacillus thuringiensis*-Susceptible *Heliothis virescens Moths* (Lepidoptera: Noctuidae): Journal of Economic Entomology 103 (3), 861-868 (2010). DOI:10.1603/EC09232.

Boerjan, W., Pilate, G., Morreel, K., Messens, E., Baucher, M., Van Doorsselaere, J., Chen, C., Meyermans, H., Pollet, B., Lapierre, C., Jouanin, L., Leplé, J.-C., Ralph, J., Marita, J., Guiney, E., Schulch, W., Petit-Conil, M., Halpin, C. (2003): Genetic engineering of lignin biosynthesis in poplar and effects on kraft pulping. Polyphenols 2002: Recent advances in polyphenols research. El Hadrami I et Daayf F, Eds, pp 34-49.

Bohan, D.A., Boffey, C.W.H., Brooks, D.R., Clark, S.J., Dewar, A.M., Firbank, L.G., Haughton, A.J., Hawes, C., Heard, M., May, M.J., Osborne, J.L., Perry, J.N., Rothery, P., Roy, D.B., Scott, R.J., Squire, G.R., Woiwod, I.P., Champion, G.T. (2005): Effects on weed and invertebrate abundance and diversity of herbicide management in genetically modified herbicide-tolerant winter-sown oilseed rape. Proceedings of the Royal Society, Series B 272: 463-474.

Bohanec, M., Cortet, J., Griffiths, B., Žnidařsič, M., Debeljak, M., Caul, S., Thompson, J., Krogh, P.H. (2007): A qualitative multi-attribute model for assessing the impact of cropping systems on soil quality. Pedobiologia 51: 239-250. DOI:10.1016/j.pedobi.2007.03.006.

Bøhn, T., Primicerio, R., Hessen, D., Traavik, T. (2008): Reduced fitness of Daphnia magna fed a Bt-transgenic maize variety. Archives of Environmental Contamination and Toxicology, 55: 584-592.

Bøhn, T., Traavik, T., Primicerio, R. (2010): Demographic responses of Daphnia magna fed transgenic Bt-maize. Ecotoxicology 19, 419-430.

Bolin, P.C., Hutchison, W.D., Andow, D.A. (1999): Long-term selection for resistance to *Bacillus thuringiensis* Cry1Ac endotoxin in a Minnesota population of European corn borer (Lepidoptera: Crambidae). Journal of Economic Entomology 92: 1021-1030.

Bonny, S. (2008): Genetically modified glyphosate-tolerant soybean in the USA: adoption factors, impacts and prospects. A review. Agronomy for Sustainable Development 28, 21-32.

Bonny, S. (2011): Herbicide-tolerant transgenic soybean over 15 years of cultivation: pesticide use, weed resistance, and some economic issues. The case of the USA. Sustainability 3, 1302-1322.

Borggard, O.K., Gimsing, A.L. (2008): Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Management Science, 64: 441-456.

Bourguet, D. (2004): Resistance to *Bacillus thuringiensis* toxins in the European corn borer: what chance for Bt-maize. Physiological Entomology 29: 251-256. DOI:10.1111/j.0307-6962.2004.00391.x.

Bourguet, D., Chaufaux, J., Micoud, A., Delos, M., Naibo, B., Bombarde, F., Marque, G., Eychenne, N., Pagliari, C. (2002): *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (Zea mays). Environmental Biosafety Research 1: 49-60. DOI:10.1051/ebr:2002005.

Bourguet, D., Chaufaux, J., Séguin, M., Buisson, C., Hinton, J.L., Stodola, T.J., Porter, P., Cronholm, G., Buschman, L.L., Andow, D.A. (2003): Frequency of alleles conferring resistance to Bt maize in French and US corn belt populations of the European corn borer, *Ostrinia nubilalis*. Theor. Appl. Genet. 106: 1225-1233.

Brammal, R.A. and Higgins V.J. (1988): The effect of glyphosate on resistance of tomato to Fusarium crown and root rot disease and the formation of host structural defensive barriers. Can. J. Bot. 66: 1547-1555.

Bravo, A., Gill, S.S., Soberón. M. (2007): Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicology. 49: 423-35.

Bravo, A., Gómez, I., Conde, J., Munoz-Garay, C., Sánchez, J., Miranda, R., Zhuang, M., Gill, S.S., Soberón, M. (2004): Oligomerization triggers binding of a *Bacillus thuringiensis* Cry1Ab pore-forming toxin to aminopeptidase N receptior leading to insertion into membrane microdomains. Biochimica et Biophysica Acta 167: 38-46. DOI:10.1016/j.bbamem.2004.08.013.

Bravo, A. and Soberón, M. (2008): How to cope with insect resistance to Bt toxins? Trends in Biotechnology, 26: 573-579.

Brigulla, M., Wackernagel, W. (2010): Molecular aspects of gene transfer and foreign DNA acquisition in prokaryotes with regard to safety issues. Applied Microbiology and Biotechnology 86, 1027-1041. DOI:10.1007/s00253-010-2489-3.

Brimner, T.A., Gallivan, G.J., Stephenson, G.R. (2005): Influence of herbicide-resistant canola on the environmental impact of weed management. Pest Management Science 61, 47-52.

Brink Van den, L., Bus, C.B., Franke, A.C., Groten, J.A.M., Lotz, L.A.P., Timmer, R.D., van de Wiel, C.C.M. (2010): Inventory of observed unexpected environmental effects of genetically modified crops. Applied Plant Research CGM 2010-08, PPO no. 3250165700.

Broderick, N.A., Raffa, K.F., Handelsman, J. (2006): Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proceedings of the National Academy of Sciences of the United States of America, 103: 15196-15199.

Broderick, N.A., Robinson, C.J., McMahon, M.D., Holt, J., Handelsman, J., Raffa, K.F. (2009): Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. BMC Biology, 7: 1-9.

Brookes, G., Barfoot, P. (2005): Global impact of biotech crops: socio-economic and environmental effects in the first ten years of commercial use. AgBioForum 9, 139-151.

Brookes, G., Barfoot, P. (2005): GM crops: The global economic and environmental impact – the first nine years 1996-2004. AgBioForum 8: 187-196.

Brooks, D.R, Bohan, D.A., Champion, G.T., Haughton, A.J., Hawes, C., Heard, M.S., Clark, S.J., Dewar, A.M., Firbank, L.G., Perry, J.N., Rothery, P., Scott, R.J., Woiwod, I.P., Birchall, C., Skellern, M.P., Walker, J.H., Baker, P., Bell, D., Browne, E.L., Dewar, A.J.G., Fairfax, C.M., Garner, B.H., Haylock, L.A., Horne, S.L., Hulmes, S.E., Mason, N.S., Norton, L.R., Nuttall, P., Randle, Z., Rossall, M.J., Sands, R.J.N., Singer, E.J., Walker, M.J. (2003): Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates. Philosophical Transactions of the Royal Society, B 358: 1847-1862. DOI:10.1098/ rstb.2003.1407.

Brower, L.P., Taylor, O.R., Williams, E.H. (2011b) Response to Davis: choosing relevant evidence to assess monarch population trends. Insect Conservation and Diversity. DOI: doi: 10.1111/j.1752-4598.2011.00176.x.

Brower, L.P., Taylor, O.R., Williams, E.H., Slayback, D.A., Zubieta, R.L., Ramirez, M.I. (2011a) Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? Insect Conservation and Diversity. DOI: 10.1111/j.1752-4598.2011.00142.x.

Brusetti, L., Francia, P., Bertolini, C., Pagliuca, A., Borin, S., Sorlini, C., Abruzzese, A., Sacchi, G., Viti, C., Giovannetti, L., Giuntini, E., Bazzicalupo, M., Daffonchio, D. (2004): Bacterial communities associated with the rhizosphere of transgenic Bt 176 maize (*Zea mays*) and its non transgenic counterpart. Plant and Soil 266: 11-21. doi:10.1007/s11104-005-5399-x.

Büchs, W., Raubuch, M., Prescher, S., Behr, K., Müller, A., Roose, K. (2005): Impact of Ostrinia-resistant Bt-maize on microbial and invertebrate decomposer communities in soil. IOBC meeting on ecological impacts on GMOs. Lleida, Spain, June 1-3 2005. Proceedings of the IOBC meeting on ecological impacts on GMOs.

Büchs, W., Schlein, O., Prescher, S. (2008): Performance of decomposers and predators under the influence of Diabrotica-resistant Bt-maize (Cry3Bb1) and conventional cultivars. Verhandlungen der Gesellschaft für Ökologie 38, 702.

Bückmann, H., Petersen, J., Schlinker, G., Märländer, B. (2000): Weed Control in Genetically Modified Sugar Beet – Two Years Experiences of a Field Trial Series in Germany. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 17: 353-62.

Burg von, S., Müller, C.B., Romeis, J. (2010): Transgenic disease-resistant wheat does not affect the clonal performance of the aphid *Metopolophium dirhodum* Walker, Basic and Applied Ecology.

Burg von, S., Veen van, F.J.F., Alvarez-Alfageme, F., Romeis, J. (2011): Aphid – parasitoid community structure on genetically modified wheat. Biology Letters. DOI:10.1098/rsbl.2010.1147.

Burgess, E.P.J., Lövei, G.L., Malone, L.A., Nielsen, I.W., Gatehouse, H.S., Christeller, J.T. (2002): Prey-mediated effects of the protease inhibitor aprotinin on the predatory carabid beetle Nebria brevivollis. Journal of Insect Physiology 48: 1093-1101.

Burgio, G., Dinelli, G., Marotti, I., Zurla, M., Bosi, S., Lanzoni, A. (2010): Bt-toxin uptake by the non-target herbivore, *Myzus persicae* (Hemiptera: Aphididae), feeding on transgenic oilseed rape in laboratory conditions. Bulletin of Entomological Research. DOI:10.1017/S0007485310000441.

Burke, J.M. and Rieseberg, L.H. (2003): Fitness effects of trangenic disease in sunflowers. Science 300: 1250.

Busov, V.B., Brunner, A.M., Meilan, R., Filichkin, S., Ganio, L., Gandhi, S. et al., (2005b): Genetic transformation: a powerful tool for dissection of adaptive traits in trees. New Phytologist 167: 9-18.

Busov, V.B., Fladung, M., Groover, A., Strauss, S. (2005a): Insertional Mutagenesis in *Populus*: Relevance and Feasibility. Tree Gene Genomes 1, 135-142.

Busse, M.D., Ratcliff, A.W., Shestak, C.J., Powers, R.F. (2001): Glyphosate toxicity and the effects of long term vegetation control on soil microbial communities. Soil Biology & Biochemistry 33: 1777-1789.

Butler, S.J., Boccaccio, L., Gregory, R.D., Vorisek, P., Norris, K. (2010): Quantifying the impact of land-use change to European farmland bird populations. Agriculture, Ecosystems and Environment 137: 348-357. DOI:10.1016/j.agee.2010.03.005.

Butler, S.J., Vickery, J.A., Norris, K. (2007): Farmland biodiversity and the footprint of agriculture. Science 315: 381-384. DOI:10.1126/science.1136607.

Calcagno, V., Bonhomme, V., Thomas, Y., Singer, M.C., Bourguet, D. (2010): Divergence in behaviour between the European corn borer, *Ostrinia nubilalis*, and its sibling species *Ostrinia* *scapulalis*: adaptation to human harvesting? Proceedings of the Royal Society B. DOI:10.1098/rspb.2010.0433.

Calvitti, M., Moretti, R., Lampazzi, E., Bellini, R., Dobson, S.L. (2010): Characterization of a New *Aedes albopictus* (Diptera: Culicidae)-*Wolbachia pipientis* (Rickettsiales: Rickettsiaceae) Symbiotic Association Generated by Artificial Transfer of the wPip Strain From *Culex pipiens* (Diptera: Culicidae): Journal of Medical Entomology 47 (2), 179-187. DOI:10.1603/ME09140.

Campbell, I.D. (1999): Quaternary pollen taphonomy: examples of differential redeposition and differential preservation. Palaeogeography, Palaeoclimatology, Palaeoecology 149, 245-256.

Campbell, L.G., Snow, A.A. (2007): Competition alters life history and increases the relative fecundity of crop-wild radish hybrids (*Raphanus* spp.). New Phytologist 173: 648-660. DOI:10.1111/j.1469-8137.2006.01941.x.

Campbell, L.G., Snow, A.A., Ridley, C.E. (2006): Weed evolution after crop gene introgression: Greater survival and fecundity of hybrids in a new environment. Ecology letters 9: 1198-1209.

Canadian Food Inspection Agency (2004): Assessment criteria for determining environmental safety of plants with novel traits. Directive 94-08 (Canadian Food Inspection Agency, Government of Canada, Ottawa, Ontario).

Candolfi, M.P., Brown, K., Grimm, C., Reber, B., Schmidli, H. (2004): A faunistic approach to assess potential side-effects of genetically modified Bt-corn on non-target arthropods under field conditions. Biocontrol Science and Technology 14: 129-170. DOI:10.1080/09583150310001655701.

Carpenter, J.E. (2010): Peer-reviewed surveys indicate positive impact of commercialized GM crops. Nature Biotechnology 29 (4), 319-321.

Carpenter, J.E. (2011): Impacts of GM crops on biodiversity. GM Crops 2 (1), 1-17.

Carrillo, L., Martinez, M., Varez-Alfageme, F., Castanera, P., Smagghe, G., Diaz, I., Ortego, F. (2010): A barley cysteine-proteinase inhibitor reduces the performance of two aphid species in artificial diets and transgenic Arabidopsis plants. Transgenic Research. DOI:10.1007/s11248-010-9417-2.

Carstens, K., Anderson, J., Bachman, P., De Shrijver, A., Dively, G., Federici, B., Hamer, M., Gielkens, M., Jensen, P., Lamp, W., Rauschen, S., Ridley, G., Romeis, J., Waggoner, A. (2011): Genetically modified crops and aquatic ecosystems: Considerations for environmental risk assessment and non-target organism testing. Transgenic Research: DOI 10.1007/s11248-011-9569-8.

Carter, M.E., Villani, M.G., Allee, L.L., Losey, J.E. (2004): Absence of non-target effects of two *Bacillus thuringiensis* coleopteran active δ-endotoxins on the bulb mite, Rhizoglypus robini (Claparède) (Acari, Acaridae). Journal of Applied Entomology 128: 56-63. DOI:10.1046/j.1439-0418.2003.00788.x.

Castaldini, M., Turrini, A., Sbrana, C., Benedetti, A., Marchionni, M., Mocali, S., Fabiani, A., Landi, S., Santomassimo, F., Pietrangeli, B., Nuti, M.P., Miclaus, N., Giovannetti, M. (2005): Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. Applied and Environmental Microbiology 71: 6719-6729. DOI:10.1128/AEM.71.11.6719-6729.2005.

Castellazzi, M.S., Matthews, J., Angevin, F., Sausse, C., Wood, G.A., Burgess, P.J., Brown, I., Conrad, K.F., Perry, J.N. (2010): Simulation scenarios of spatio-temporal arrangement of crops at the landscape scale. Environmental Modelling & Software. DOI:10.1016/j.envsoft.2010.04.006.

Catangui, M.A., Berg, R.K. (2006): Western bean cutworm, Striacosta albicosta (Smith (Lepidoptera: Noctuidae), as a potential pest of transgenic Cry1Ab Bacillus thuringiensis corn hybrids in SouthDakota. Environmental Entomology, 35: 1439-1452.

Caviness, C.E. (1966): Estimates of natural cross-pollination in Jackson soybeans in Arkansas. Crop Science 6: 211.

CERA (2010): A review of the environmental safety of the CP4 EPSPS protein, ILSI Research Foundation, Washington D.C., http://cera-gmc.org/docs/cera_publications/pub_01_2010.pdf

Cerdeira, A.L. and Duke, S.O. (2006): The current status and environmental impacts of glyphosate-resistant crops. A review. Journal of Environmental Quality 35: 1633-1658.

Cerdeira, A.L., Duke, S.O. (2007): Environmental impacts of transgenic herbicide-resistant crops. CAB Reviews 2: 26-40.

Cerdeira, A.L., Duke, S.O. (2010): Effects of gluphosate-resistant crop cultivation on soil and water quality. GM Crops 1:1, 1-9.

Chamberlain, D.E., Freeman, S.N., Vickery, J.A. (2007): The effects of GMHT crops on bird abundance in arable fields in the UK. Agriculture, Ecosystems and Environment 118: 350-356. DOI:10.1016/j.agee.2006.05.012.

Chambers, C.P., Whiles, M.R., Griffiths, N.A., Evans-White, M.A., Rosi-Marshall, E.J., Tank, J.L., Royer, T.V. (2007): Assessing the impacts of transgenic Bt corn detritus on macroinvertebrate communities in agricultural streams. North American Benthological Society 55th Annual Meeting, pp. 373.

Chambers, C.P., Whiles, M.R., Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Griffiths, N.A., EvansWhite, M.A., Stojak, A.R. (2010): Responses of stream macroinvertebrates to Bt maize leaf detritus. Ecological Applications, 20 (7), 1949–1960.

Champion, G. (2011): Lessons learned from the farm scale evaluation of GMHT crops. Journal für Verbraucherschutz und Lebensmittelsicherheit. DOI:10.1007/s0003-011-0678-3.

Champion, G.T., May, M.J., Bennett, S., Brooks, D.R., Clark, S.J., Daniels, R.E., Firbank, L.G., Haughton, A.J., Hawes, C., Heard, M.S., Perry, J.N., Randle, Z., Rossall, M.J., Rothery, P., Skellern, M.P., Scott, R.J., Squire, G.R., Thomas, M.R. (2003): Crop management and agronomic context of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Proceedings of the Royal Society, Series B 358: 1801-1818. DOI:10.1098/rstb.2003.1405.

Chassy, B., Carter, C., McGloughlin, M., McHughen, A., Parrott, W., Preston, C., Roush, R., Shelton, A., Strauss, S.H. (2003): UK field-scale evaluations answer wrong questions. Nature Biotechnology, 21: 1429-143.

Chapman, M.A., Burke, J.M. (2006): Tansley review – Letting the gene out of the bottle: The population genetics of genetically modified crops. New Phytologist 170: 429-443. DOI:10.1111/j.1469-8137.2006.01710.x.

Chaufaux, J., Séguin, M., Swanson, J.J., Bourguet, D., Siegfried, B.D. (2001): Chronic exposure of the European corn borer (Lepidoptera: Crambidae) to Cry1Ab *Bacillus thuringiensis* toxin. Journal of Economic Entomology 94: 1564-1570.

Chen, L., Cui, J., Ma, W., Changying, N., Chaoliang, L. (2010): Pollen from Cry1Ac/CPTI-transgenic cotton does not affect the pollinating beetle *Haptoncus luteolus*. Journal of Pest Science. DOI:10.1007/s10340-010-0319-0.

Chèvre, A.M., Ammitzbøll, H., Breckling, B., Dietz-Pfeilstetter, A., Eber, F., Fargue, A., Gomez-Campo, C., Jenczewski, E., Jorgensen, R., Lavigne, C., Meier, M.S., Nijs, H.C.M. den, Pascher, K., Séguin-Swartz, G., Sweet, J., Stewart Jr., C.N., Warwick, S. (2004): A review on interspecific gene flow from oilseed rape to wild relatives. In: Nijs, H.C.M. den, Bartsch, D., Sweet, J. (Eds); Introgression from genetically modified plants into wild relatives: 203-218. CABI Publishing.

Chèvre, A.M., Eber, F., Baranger, A., Renard, M. (1997): Gene flow from transgenic crops. Nature 389: 924. DOI:10.1038/40054.

Claessen, D., Gilligan, C.A., Lutman, P.J.W., Van Den Bosch, F. (2005a): Which traits promote persistence of feral GM crops? Part 1: Implications of environmental stochasticity. Oikos 110 (1), 20.

Claessen, D., Gilligan, C.A., Van Den Bosch, F. (2005b): Which traits promote persistence of feral GM crops? Part 2: Implications of metapopulation structure. Oikos 110 (1), 30.

Clark, B.W., Prihoda, K.R., Coats, J.R. (2006): Subacute effects of transgenic Cry1Ab *Bacillus thuringiensis* corn litter on the isopods Trachelipus rathkii and Armadillidium nasatum. Environmental Toxicology and Chemistry 25: 2635-2661.

Clarke, M. (2012): Of Rio, rainforests and Richmond Park. Birds 24, 2, 4-5.

Clive, J. (2008): Global Status of Commercialized Biotech/GM Crops: 2008. ISAAA Brief No. 39. ISAAA: Ithaca, NY. http://www.isaaa.org/resources/publications/briefs/39/default.html

COGEM report CGM/061024-02 (2010): New techniques in plant biotechnology.

Coley, P.D., Bryant, J.P., Chapin, F.S. (1985): Resource availability and plant antiherbivore defense. Science 230: 895-899. DOI:10.1126/science.230.4728.895.

Coll, A., Nadal, A., Collado, R., Capellades, G., Kubista, M., Messeguer, J., Pla, M. (2010): Natural variation explains most transcriptomic changes among maize plants of MON810 and comparable non-GM varieties subjected to two N-fertilization farming practices. Plant Molecular Biology 73, 349-362. DOI:10.1007/s11103-010-9624-5.

Coll, A., Nadal, A., Rossignol, M., Puigdomenech, P., Pla, M. (2010): Proteomic analysis of MON810 and comparable non-GM maize varieties grown in agricultural fields. Transgenic Research. DOI:10.1007/s11248-010-9453-y.

Collier, M.J., Mullins, E. (2011): The CINMA Index: Assessing the potential impact of GM crop management across a heterogeneous landscape. Environmental Biosafety Research. DOI:10.1051/ebr/2011102.

Cominelli, E., Tonelli, C. (2010): Transgenic crops coping with water scarcity. New Biotechnology 27, 473-477. DOI:10.1016/j.nbt.2010.08.005.

Conner, A.J. (1997): Biosafety assessment of transgenic potatoes: Environmental monitoring and food safety evaluation Proceedings of the 3rd International Symposium on the Biosafety Results of Field Tests of Genetically Engineered Plants and Microorganisms: 245-262.

Cook, S.M., Khan, Z.R., Pickett, J.R. (2007): The Use of Push-Pull Strategies in Integrated Pest Management. Annual Review of Entomology 52, 375-400.

Cortet, J., Andersen, M.N., Caul, S., Griffiths, B., Joffre, R., Lacroix, B., Sausse, C., Thompson, J., Krogh, P.H. (2006): Decomposition processes under Bt (*Bacillus thuringiensis*) maize: Results of a multi-site experiment. Soil Biology & Biochemistry 38: 195-199. DOI:10.1016/j.soilbio.2005.04.025.

Cortet, J., Griffiths, B., Bohanec, M., Demšar, D., Andersen, M.N., Caul, S., Birch, A.N.E., Pernin, C., Tabone, E., Vaufleury, A. de, Ke, X., Krogh, P.H. (2007): Evaluation of effects of transgenic Bt maize on microarthropods in a European multi-site experiment. Pedobiologia 51: 207-218. DOI:10.1016/j.pedobi.2007.04.001.

Cox, W.J., Hahn, R.R., Stachowski, P.J. (2006): Time of weed removal with glyphosate affects corn growth and yield components. Agronomy Journal, 98: 349-353.

Coyette, B., Tencalla, F., Brants, I., Fichet, Y., Rouchouze, D. (2002): Effect of introducing glyphosate-tolerant sugar beet on pesticide usage in Europe. Pesticide Outlook, 13: 219-223.

Crawley, M.J. and Brown, S.L. (1995): Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. Proceedings of the Royal Society, Series B 259: 49-54.

Crawley, M.J. and Brown, S.L. (2004): Spatially structured population dynamics in feral oilseed rape. Proceedings of the Royal Society, Series B 271: 1909-1916.

Crawley, M.J., Brown, S.L., Hails, R.S., Kohn, D.D., Rees, M. (2001): Transgenic crops in natural habitats. Nature 409: 682-683.

Crawley, M.J., Hails, R.S., Rees, M., Kohn, D.D., Buxton, J. (1993): Ecology of transgenic oilseed rape in natural habitats. Nature 363: 620-623. DOI:10.1038/363620a0.

Crespo, A.L., Spencer, T.A., Tan, S.Y., Siegfried, B.D. (2010): Fitness costs of Cry1Ab resistance in a field-derived strain of *Ostrinia nubilalis* (Lepidoptera: Crambidae): Journal of Economic Entomology 103 (4), 1386-1393. DOI:10.1603/EC09158.

Crickmore, N. (2005): Using worm to better understand how *Bacillus thuringiensis* kills insects. Trends in Microbiology, 13: 347-350.

Culpepper, A.S., Webster, T.M., Sosnoskie, L.M., York, A.C. (2010): Glyphosate resistant Palmer amaranth in the United States. In: Nandula VK (Ed), Glyphosate Resistance in Crops and Weeds: History, Development, and Management, John Wiley & Sons, Inc., New York, pp 195-212.

Cureton, A.N., Newbury, H.J., Raybould, A.F., Ford-Lloyd, B.V. (2006): Genetic structure and gene flow in wild beet populations: the potential influence of habitat on transgene spread and risk assessment. Journal of Applied Ecology 43: 1203-1212. DOI:10.1111/j.1365-2664.2006.01236.x.

Dalecky, A., Ponsard, S., Bailey, R.I., Pélissier, C., Bourguet, D. (2006): Resistance evolution to Bt crops: predispersal mating of European corn borers. PLoS Biol. 4: 1048-1057 (e181).

Darmency, H., Lefol, E., Fleury, A. (1998): Spontaneous hybridizations between oilseed rape and wild radish. Molecular Ecology 7: 1467-1473.

Darmency, H. and Richard-Molard, M, (2008): Gene flow in sugar beet production fields. ISB News Report (April 2008).

Darmency, H., Vigouroux, Y., Gestat Garambé, T. de, Richard-Molard, M., Muchembled, C. (2007): Transgene escape in sugar beet production fields: data from six years farm scale monitoring. Environmental Biosafety Research 6: 197-206. DOI:10.1051/ebr:2007007.

Dauer, J.T., Luschei, E.C., Mortensen, D.A. (2009): Effects of landscape composition on spread of an herbicide-resistant weed. Landscape Ecology 24, 735-747.

Davis, A.K. (2011): Are migratory monarchs really declining in eastern North America? Examining evidence from two fall census programs. Insect Conservation and Diversity, DOI: 10.1111/j.1752-4598.2011.00158.x.

Davison, J. (1999): Genetic exchange between bacteria in the environment. Plasmid 42: 73-91.

Davison, J. (2010): GM Plants: Science, politics and EC regulations. Plant Science. DOI:10.1016/ j.plantsci.2009.12.005.

Dechaume-Moncharmont, F.X., Azzouz, H., Pons, O., Pham-Delègue, M.H. (2005): Soybean proteinase inhibitor and the foraging strategy of free flying honeybees. Apidologie 36: 421-430. DOI:10.1051/apido:2005031.

DEFRA (2005): Secretary of State Margaret Beckett's statement on GM policy, http://webarchive.nationalarchives.gov.uk/20080306073937/http://www.defra.gov.uk/environment/gm/fse/

De Jong, T.J. (2010): General surveillance of genetically modified plants in the EC and the need for controls. Journal für Verbraucherschutz und Lebensmittelsicherheit 5, 181-183. DOI:10.1007/s00003-009-0547-5.

Delannay, X., Bauman, T.T., Beighley, D.H., Buettner, M.J., Coble, H.D., DeFelice, M.S., Derting, C.W., Diedrick, T.J., Friffin, J.L., Hagood, E.S., Hancock, F.G., Hart, S.E., LaVallee, B.J., Loux, M.M., Lueschen, W.E., Matson, K.W., Moots, C.K., Murdock, E., Nickell, A.D., Owen, M.D.K, Paschal, E.H., Prochaska, L.M., Raymond, P.J., Reynolds, D.B., Rhodes, W.K., Roeth, F.W., Sprankle, P.L., Tarochione, L.J., Tinius, C.N., Walker, R.H., Wax, L.M., Weigelt, H.D., Padgette, S.R. (1995): Yield evaluation of a glyphosate-tolerant soybean line after treatment with glyphosate. Crop Science 35, 1461-1467.

Delos, M., Hervieu, F., Folcher, L., Micoud, A., Eychenne, N. (2007): Biological surveillance programme for the monitoring of crop pests and indicators, French devices and European approach compared. J. Consum. Protec. Food Safety 2(S1): 16-24.

Deml, R., Meise, T., Dettner, K. (1999): Effects of *Bacillus thuringiensis* d-endotoxins on food utilization, growth, and survival of selected phytophagous insects. Journal of Applied Entomology 123: 55-64.

De Schrijver, A., Devos, Y., Van den Bulcke, M., Cadot, P., De Loose, M., Reheul, D., Sneyers, M. (2007): Risk assessment of GM stacked events obtained from crosses between GM events. Trends in Food Science and Technology, 18: 101-109.

De Vaufleury, A., Kramarz, P.E., Binet, P., Cortet, J., Caul, S., Andersen, M.N., Plumey, E., Coeurdassier, M., Krogh, P.H. (2007): Exposure and effect assessments of *Bt*-maize on non-target organisms (gastropods, microarthropods, mycorrhizal fungi) in microcosms. Pedobiologia 51: 185-194.

Devare, M., Londoño-R, L.M., Thies, J.E. (2007): Neither transgenic Bt maize (MON863) nor tefluthrin insecticide adversely affect soil microbial activity or biomass: A 3-year field analysis. Soil Biology & Biochemistry 39: 2038-2047. DOI:10.1016/j.soilbio.2007.03.004.

Devos, Y., Cougnon, M., Vergucht, S., Bulcke, R., Haesaert, G., Steurbaut, W., Reheul, D. (2008): Environmental impact of herbicide regimes used with genetically modified herbicide-resistant maize. Transgenic Research 17, 1059-1077 (Erratum: 18, 315-316).

Devos, Y., Demont, M., Dillen, K., Reheul, D., Kaiser, M., Sanvido, O. (2009c): The coexistence of genetically modified (GM) and non-GM crops in the European Union. Agronomy for Sustainable Development, 29: 11-30.

Devos, Y., Demont, M., Sanvido, O. (2008): Coexistence in the EU return of the moratorium on GM crops? Nat. Biotechnol. 26: 1223-1225.

Devos, Y., De Schrijver, A., Reheul, D. (2009): Quantifying the introgressive hybridisation propensity between transgenic oilseed rape and its wild/weedy relatives. Environ. Monit. Assessm. 149: 303-322.

Devos, Y., Hails, R.S., Messéan, A., Perry, J.N., Squire, G.R. (2011): Feral genetically modified herbicide tolerant oilseed rape from seed import spills: are concerns scientifically justified? Transgenic Research. DOI:10.1007/s11248-011-9515-9.

Devos, Y., Lheureux, K., Schiemann, J. (2010): Regulatory oversight and safety assessment of plants with novel traits. In: Kempken et al., (eds) Genetic modification of plants – agriculture, horticulture & forestry, Springer Verlag, 64 (4): 553-574.

Devos, Y., Reheul, D., Schrijver, A. de (2005): The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization. Environmental Biosafety Research 4: 71-87. DOI:10.1051/ebr:2005013.

Devos, Y., Reheul, D., Schrijver, A. de, Cors, F., Moens, W. (2004): Management of herbicide-tolerant oilseed rape in Europe: a case study on minimizing vertical gene flow. Environmental Biosafety Research 3: 135-148.

De Vries, J., Herzfeld, T., Wackernagel, W. (2004): Transfer of plastid DNA from tobacco to the soil bacterium *Acinetobacter* sp. by natural transformation. Molecular Microbiology 53: 323-334. DOI:10.1111/j.1365-2958.2004.04132.x.

De Vries, J., Meier, P., Wackernagel, W. (2001): The natural transformation of the soil bacteria Pseudomonas stutzeri and *Acinetobacter* sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. FEMS Microbiology Letters 195: 211-215.

De Vries, J., Wackernagel, W. (2002): Integration of foreign DNA during natural transformation of *Acinetobacter* sp. by homology-facilitated illegitimate recombination. Proceedings of the National Academy of Sciences of the USA 99: 2094-2099.

De Vries, J. and Wackernagel, W. (2005): Microbial horizontal gene transfer and the DNA release from transgenic crop plants. Plant and Soil 266: 91-104. DOI:10.1007/s11104-005-4783-x.

Dewar, A.M. (2010): Gm Glyphosate-Tolerant Maize in Europe can help Alleviate the global food shortage. Pest Management Science, 55-63. DOI:10.1564/21apr02.

Dewar, A.M., May, M.J., Woiwod, I.P., Haylock, L.A., Champion, G.T., Garner, B.H., Sands, R.J.N., Qi, A., Pidgeon, J.D. (2003): A novel approach to the use of genetically modified herbicide tolerant crops for environmental benefit. Proceedings of the Royal Society B-Biological Sciences, 270: 335-340.

Dhurua, S., and Gujar, G.T., (2011): Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), from India. Pest Manag. Sci. 67: 898-903.

Di, K., Stewart, C.N.Jr., Wei, W., Shen, B., Tang, Z.-X., Ma, K.-P. (2009): Fitness and maternal effects in hybrids formed between transgenic oilseed rape (*Brassica napus* L.) and wild brown mustard [*B. juncea* (L.) Czern et. Coss.] in the field. Pest Management Science 65:753-760.

Dicke, M., Baldwin, I.T. (2010): The evolutionary context for herbivore-induced plant volatiles: beyond the ‘cry for help’. Trends in Plant Science Vol.15 No.3.

Dijk, H. van, Boudry, P., McCombie, H., Vernet, P. (1997): Flowering time in wild beet (*Beta vulgaris* spp. *maritima*) along a latitudinal cline. Acta Oecologica 18: 47-60.

Dill, G.M. (2005): Glyphosate-resistant crops: history, status and future. Pest Management Science, 61: 219-224.

Dillen, K., Mitchell, P.D., Van Looy, T., Tollens, E. (2010a): The western corn rootworm, a new threat to European agriculture: opportunities for biotechnology. Pest Management Science. DOI:10.1002/ps.1966.

Dillen, K., Mitchel, P.D., Tollens, E. (2010b): On the competitiveness of *Diabrotica virgifera virgifera* damage abatement strategies in Hungary: a bio-economic approach. Journal of Applied Entomology, 1-14. DOI:10.1111/j.1439-0418.2009.01454.x.

Dively, G., Rose, R., Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Calvin, D.D., Russo, J.M., Anderson, P.L. (2004): Effects on Monarch Butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab-expressing corn during anthesis. Environmental Entomology 33: 1116-1125.

Dively, G.P. (2005): Impact of transgenic VIP3A x Cry1Ab Lepidopteran-resistant field corn on the nontarget arthropod community. Environmental Entomology 34: 1267-1291.

Dolezel, M., Heissenberger, A., Gaugitsch, H. (2005): Ecological effects of genetically modified maize with insect resistance and/or herbicide tolerance. Forschungsberichte der Sektion IV d. Österreichischen Umweltbundesamtes 6/2005. www.bmgfj.gv.at/cms/site/attachments/5/6/2/CH0810/CMS1134457515326/cms1200662494442_literaturstudie_mais_endbericht.pdf

Donald, P.F. et al., (2006): Further evidence of continent-wide impacts of agricultural intensification on European farmland birds, 1990-2000. Agriculture, Ecosystems and Environmental 116: 189-196.

Donald, P.F., Sanderson, F.A., Burfield, I.J., van Bommel, F.P.J. (2006): Further evidence of continent-wide impacts of agricultural intensification on European farmland birds, 1990-2000. Agriculture, Ecosystems and Environmental 116: 189-196.

Donegan, K.K., Palm, C.J., Fieland, V.J., Porteous, L.A., Ganio, L.M., Schaller, D.L., Bucao, L.Q., Seidler, R.J. (1995): Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. kurstaki endotoxin. Applied Soil Ecology 2: 111-124. DOI:10.1016/0929-1393(94)00043-7.

Donnarumma, F., Fladung, M., Giannini, R., Altosaar, I., Biricolti, S., Vettori, C. (2007a): Risks Analyses In cry Transgenic Poplar. IUFRO: Tree Biotechnology. (Ponta Delgada, Azores, Protugal, 3-8 Giugno, 2007). Proceedings: http://www.itqb.unl.pt/iufro2007/abstracts/Session%20III%20-%20webpage.pdf

Donnarumma, F., Fladung, M., Giannini, R., Altosaar, I., Biricolti, S., Vettori, C. (2007b): Risks analyses in cry transgenic poplar. Invited speaker at Workshop: Poplar Ecophisiology, Biology and Genetics: state of art of the research in Italy. (Area della Ricerca CNR, Sesto Fiorentino, Italy, 25 Maggio 2007): Published on-line: http://www.sisef.it/forest@/pdf/Giannini_479.pdf, http://www.sisef.it/sisef/poplar.php

Donnarumma, F., Fladung, M., Giannini, R., Altosaar, I., Biricolti, S., Vettori, C. (2008): Preliminary results of different cry transgenic poplar lines. FISV, 10th Annual Congress (Riva del Garda, 24-27 Settembre). Proceedings: http://fisv2008.azuleon.org/ProgrammePosters_2008_SList.php?ps=7.6

Dorhout, D.L., Rice, M.E. (2010): Intraguild competition and enhanced survival of western bean cutworm (Lepidoptera: Noctuidae) on transgenic Cry1Ab (MON810) *Bacillus thuringiensis* corn. Journal of Economic Entomology 103 (1), 54-62. DOI:10.1603/EC09247.

Douville, M., Gagné, F., André, C., Blaise, C. (2009): Occurrence of the transgenic corn cry1Ab gene in freshwater mussels (*Elliptio complanata*) near corn fields: evidence of exposure by bacterial ingestion. Ecotox. Environ. Safety 72: 17-25.

Douville, M., Gagné, F., Blaise, C., André, C. (2007): Occurrence and persistence of *Bacillus thuringiensis* (Bt) and transgenic Bt corn cry1Ab gene from an aquatic environment. Ecotoxicology and Environmental Safety 66: 195-203. DOI:10.1016/j.ecoenv.2006.01.002.

Douville, M., Gagné, F., Masson, L., McKay, J., Blaise, C. (2005): Tracking the source of *Bacillus thuringiensis* Cry1Ab endotoxin in the environment. Biochem. System. Ecol. 33: 219-232.

Down, R.E., Ford, L., Woodhouse, S.D., Davison, G.M., Majerus, M.E.N., Gatehouse, J.A., Gatehouse, M.R. (2003): Tritrophic interactions between transgenic potato expressing snowdrop lectin (GNA); an aphid pest (peach-potato aphid; Myzus persicae) (Sulz.) and a beneficial predator (2-spot ladybird, Adalia bipunctata L.). Transgenic Research 12: 229-241.

Downes, S., Mahon, R., Olsen, K. (2007): Monitoring and adaptive resistance management in Australia for Bt-cotton: Current status and future challanges. Journal of Invertebrate Pathology 95: 208-213. DOI:10.1016/j.jip.2007.03.010.

Downes, S., Parker, T., Mahon, R. (2010): Incipient Resistance of *Helicoverpa punctigera* to the Cry2Ab Bt Toxin in Bollgard IIH Cotton. Plos One.

Droge, J., Pühler, A., Selbitschka, W. (1998): Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern. Journal of Biotechnoloy 64: 75-90. DOI:10.1016/S0168-1656(98)00105-9.

Duan, J.J., Jiang, C., Head, G.P., Bhatti, M.A., Ward, D.P., Levine, S.L., Nickson, T.E., Nemeth, M.A. (2006): Statistical power analysis of a 2-year field study and design of experiments to evaluate non-target effects of genetically modified *Bacillus thuringiensis* corn. Ecological Entomology 31: 521-531. DOI:10.1111/j.1365-2311.2006.00811.x.

Duan, J.J., Marvier, M., Huesing, J., Dively, G., Huang, Z.Y. (2008): A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). Public Library of Science 3: e1415. DOI:10.1371/journal.pone.0001415.

Dubelman, S., Ayden, B.R., Bader, B.M., Brown, C.R., Jiang, C., Vlachos, D. (2005): Cry1Ab protein does not persist in soil after 3 years of sustained Bt corn use. Environmental Entomology 34: 915-921.

Duke, S.O. (2005): Taking stock of herbicide-resistant crops ten years after introduction. Pest Manage-ment Science 61, 211-218.

Duke, S.O., Powles, S.B. (2008 a): Glyphosate-resistant weeds and crops. Pest Management Science 64, 317-318.

Duke, S.O. and Powles, S.B. (2008b): Glyphosate: a once-in-a-century herbicide. Pest Management Science, 64: 319-325.

Dutton, A., Romeis, J., Bigler, F. (2003): Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-Maize expressing Cry1Ab as a case study. BioControl 48: 611-636.

Dutton, A., Romeis, J., Bigler, F. (2005): Effects of Bt maize expressing Cry1Ab and Bt spray on *Spodoptera littoralis*. Entomol. Experim. Appl. 114: 161-169.

Eastham, K. and Sweet, J. (2002): Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer. Eurpean Environment Agency (EEA), Environmental Issue Report, 28: 75 pp.

EC (2006): Questions and Answers on the finding of unauthorised GM rice on the US market. http://europa.eu/rapid/pressReleasesAction.do?reference=MEMO/06/310

EC (2008): Letter from the European Commission to EFSA on the environmental risk assessment of herbicide tolerant plants – interplay between Directive 2001/18/EC and Directive 91/414/EEC (Ref ENV/B3/AA/JH/YK/gm D(2008)ARES(2008)25125), http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/gmo_response_european_commission_en.pdf

Eckert, J.W. (1988): Historical development of fungicide resistance in plant pathogens. In: Delp, C.J. (Ed); Fungicide Resistance in North America: 1-3. APS Press. St. Paul.

Eckert, J., Schuphan, I., Hothorn, L.A., Gathmann, A. (2006): Arthropods on maize ears for detecting impacts of Bt maize on nontarget organisms. Environmental Entomology 35: 554-560.

Econopouly, B.F., McKay, J.K., Westra, P., Lapitan, N.L.V., Chapman, P.L., Byrne, P. F. (2011): Backcrossing provides an avenue for gene introgression from wheat to jointed goatgrass (Aegilops cylindrica) in the U.S. Great Plains. Weed Science , 59, 188-194.

EcoStrat (2000): Review on non target organisms and Bt-plants. Hilbeck A, Meier M, Raps A. GmbH, Zurich, Switzerland (for Greenpeace International, Amsterdam). http://www.greenpeace.org/international/Global/international/planet-2/report/2000/3/review-on-non-target-organisms.pdf

EFSA (2004): Guidance document on the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99: 1-94.

EFSA (2005a): Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507, for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. The EFSA Journal, 181: 1-33. http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/op_gm08_ej181_1507_opinion_doc1_2en1.pdf

EFSA (2005b): Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/F/96/05.10) for the placing on the market of insect resistant genetically modified maize Bt11, for cultivation, feed and industrial processing, under Part C of Directive 2001/18/EC from Syngenta Seeds. The EFSA Journal, 213: 1-33. http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej213_bt11maize_cultivation_en10.pdf

EFSA (2006a): Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (Question No EFSA-Q-2003-005). The EFSA Journal (2006) 99, 1-100.

EFSA (2006b): Opinion of the Scientific Panel on Genetically Modified Organisms on the Post Market Environmental Monitoring (PMEM) of genetically modified plants, EFSA J. 319, 1-27, http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej319_pmem_en,0.pdf

EFSA (2006c): Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/SE/96/3501) for the placing on the market of genetically modified potato EH92-527-1 with altered starch composition, or cultivation and production of starch, under Part C of Directive 2001/18/EC from BASF Plant Science. The EFSA Journal 323: 1-20.

EFSA (2007a): Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants containing stacked transformation events. The EFSA Journal 512: 1-5.

EFSA (2007b): Statement of the Scientific Panel on Genetically Modified Organisms on the safe use of the nptII antibiotic resistance marker gene in genetically modified plants. http://www.efsa.europa.eu/cs/BlobServer/Statement/gmo_statement_nptII_,0.pdf

EFSA (2007c): Minutes of the 37th plenary meeting of the Scientific Panel on Genetically Modified Organisms held on 22-23 November 2007 in Brussels, Belgium (adopted on 18 December 2007). http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/GMO_Minutes_37th_plenmeet.pdf

EFSA (2007d): Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-UK-2004-04) for the placing on the market of glufosinate tolerant genetically modified rice LLRICE62 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 from Bayer CropScience GmbH. The EFSA Journal 588, 1-25.

EFSA (2008a): Working Document of the GMO Panel on the interplay between Directive 2001/18/EC (GMOs) and Directive 91/414/EEC (Plant Protection Products). http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902125247.htm

EFSA (2008b): Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the safeguard clause invoked by Austria on maize MON810 and T25 according to Article 23 of Directive 2001/18/EC. EFSA J. 891, 1-64. http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej891_austrian_safeg_clause_MON810_T25_maize_en.pdf

EFSA (2008c): Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the safeguard clause invoked by France on maize MON810 according to Article 23 of Directive 2001/18/EC and the emergency measure according to Article 34 of Regulation (EC) No 1829/2003. EFSA J. 850, 1-45. http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej850_French_safeguard_clause_on_MON810_maize_en,0.pdf

EFSA (2008d): Proceedings of the EFSAs 8th Scientific Colloquium - Environmental Risk Assessment of Genetically Modified Plants – Challenges and Approaches, 20-21 June 2007, Tabiano, Italy.

EFSA (2008e): Request from the European Commission related to the safeguard clause invoked by Hungary on maize MON810 according to Article 23 of Directive 2001/18/EC. The EFSA Journal, 756: 1-18. http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej757_greek_safeguard_clause_on_mon810_maize_en.pdf

EFSA (2008f): Request from the European Commission related to the safeguard clause invoked by Greece on maize MON810 according to Article 23 of Directive 2001/18/EC. The EFSA Journal, 757: 1-12. http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej757_greek_safeguard_clause_on_mon810_maize_en.pdf

EFSA (2009a): Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, and maize MON810 for feed use (including cultivation); and of (3) food additives and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal (2009).

EFSA (2009b): Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-NL-2005-22 and EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 cultivation and for food and feed uses, and for renewal of the authorisation of maize NK603 as existing product. The EFSA Journal (2009) 1137: 1-50.

EFSA (2009c): Scientific Opinion of the Panel on Genetically Modified Organisms (GMO) and the Panel on Biological Hazards (BIOHAZ) on a request from the European Commission on the use of antibiotic resistance genes as marker genes in genetically modified plants. The EFSA Journal, 1034: 1-81. http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOOpinions455.htm

EFSA (2009d): Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of antibiotic resistance genes as marker genes in genetically modified plants" and the Scientific Opinion of the GMO Panel on "Consequences of the opinion on the use of antibiotic resistance genes as marker genes in genetically modified plants on previous EFSA assessments of individual GM plants". The EFSA Journal 1108, 1-8.

EFSA (2010a): Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010; 8(11):1879. [111 pp.]. DOI:10.2903/j.efsa.2010.1879. Available online: www.efsa.europa.eu/efsajournal.htm

EFSA (2010b): Scientific opinion of the EFSA GMO Panel on the assessment of potential impacts of genetically modified plants on non-target organisms. The EFSA Journal 8, 1877 Available online: www.efsa.europa.eu/efsajournal.htm

EFSA (2011a): Request for an analysis of the Testbiotech letter concerning applications for authorisation of MON89034 x MON88017 maize and GHB614 cotton and for renewal of authorisation of 1507 maize. EFSA-Q-2011-01068. Available online: http://registerofquestions.efsa.europa.eu/

EFSA (2011b): Statement complementing the EFSA GMO Panel scientific opinion on maize MON 89034 x 1507 x MON 88017 x 59122 (application EFSA-GMO-CZ-2008-62), to cover all sub-combinations independently of their origin. EFSA Journal 2011;9(10):2399. [8 pp.] DOI:10.2903/j.efsa.2011.2399. Available online: www.efsa.europa.eu/efsajournal

EFSA (2011c): Scientific Opinion updating the evaluation of the environmental risk assessment and risk management recommendations on insect resistant genetically modified maize 1507 for cultivation. EFSA Journal 9: 2429. [73 pp.] DOI:10.2903/j.efsa.2011.2429. Available online: www.efsa.europa.eu/efsajournal

EFSA (2011d): Scientific Opinion on application (EFSAGMO-CZ-2008-54) for placing on the market of genetically modified insect resistant and herbicide tolerant maize MON 88017 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2011;9(11):2428. [152 pp.]doi:10.2903/j.efsa.2011.2428. Available online: www.efsa.europa.eu/efsajournal

EFSA (2011e): Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. The EFSA Journal 2316, 1-40, http://www.efsa.europa.eu/en/efsajournal/doc/2316.pdf

EFSA (2011f): Scientific Opinion on application (EFSA GMO-UK-2008-60) for placing on the market of genetically modified herbicide tolerant maize GA21 for food and feed uses,import, processing and cultivation under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2011;9(12):2480. [94 pp.] DOI:10.2903/j.efsa.2011.2480. Available online: www.efsa.europa.eu/efsajournal

EFSA (2012a): Scientific Opinion on an application (EFSA-GMO-NL-2005-24) for the placing on the market of the herbicide tolerant genetically modified soybean GTS 40-3-2 for cultivation under Regulation (EC) No 1829/2003 from Monsanto, in preparation

EFSA (2012b): Scientific Opinion on application (EFSA-GMO-NL-2009-73) for the placing on the market of insect resistant and herbicide tolerant genetically modified soybean MON 87701 × MON 89788 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 2012; 10(2):2560. [34 pp.] DOI:10.2903/j.efsa.2012.2560. Available online: www.efsa.europa.eu/efsajournal

EFSA (2012c): Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010. EFSA Journal 2012;10(4):2610. [35 pp.] DOI:10.2903/j.efsa.2012.2610. Available online: www.efsa.europa.eu/efsajournal

EFSA (2012d): Scientific Opinion on a request from the European Commission related to the emergency measure notified by France on genetically modified maize MON 810 according to Article

34 of Regulation (EC) No 1829/2003. EFSA Journal 2012;10(5):2705. [21 pp.] DOI:10.2903/j.efsa.2012.2705. Available online: www.efsa.europa.eu/efsajournal

Ehler, L.E. (1990): Environmental impact of introduced biological-control agents: implications for agricultural biotechnology. In: Division of Agricultural and Natural Resources, University of California (Eds); Risk assessment in agricultural biotechnology: proceedings of the International Conference, Davis, California, USA, August, 1988. Oakland, USA.

Ehlers, U. (2011): Interplay between GMO regulation and pesticide regulation in the EU. Journal für Verbraucherschutz und Lebensmittelsicherheit 6 (Suppl 1), S61-S64. DOI:10.1007/s00003-011-0672-9.

Eichenseer, H., Strohbehn, R., Burks, J. (2008): Frequency and severity of Western Bean Cutworm (Lepidoptera: Noctuidae) ear damage in transgenic corn hybrids expressing different Bacillus thuringiensis Cry toxins. Journal of Economic Entomology, 101: 555-563.

Eijlander, R. and Stiekema, W.J. (1994): Biological containment of potato (Solanum tuberosum): outcrossing to the related wild species Black nightshade (Solanum nigrum) and bittersweet (Solanum dulcamara). Sexual Plant Reproduction 7: 29-40.

Eizaguirre, M., Albajes, R., López, C., Eras, J., Lumbierres, B., Pons, X. (2006): Six years after the commercial introduction of Bt maize in Spain: field evaluation, impact and future prospects. Transgenic Research 15: 1-12. DOI:10.1007/s11248-005-3998-1.

Eker, S., Ozturk, L., Yazici, A., Erenoglu, B., Romheld, V., Cakmak, I. (2006): Foliar applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (Helianthus annuus L.) plants. Journal of Agricultural Food Chemistry 56: 10019-10025.

Ellstrand, N.C. (2001): When transgenes wander, should we worry? Plant Physiology 151: 1543-1545.

Ellstrand, N.C. (2003b): Current knowledge of gene flow in plants: implications for transgene flow. Philosophical Transactions of the Royal Society, B 358: 1163-1170. DOI:10.1098/rstb.2003.1299.

Ellstrand, N.C. (2003a): Dangerous liaisons? When cultivated plants mate with their wild relatives. In: Scheiner, S.M (Ed); Syntheses in Ecology and Evolution. Baltimore, MD, USA. The Johns Hopkins University Press.

Ellstrand, N.C., Heredia, S.M., Leak-Garcia, J.A., Heraty, J.M., Burger, J.C., Yao, L., Nohzadeh-Malakshah, S., Ridley, C.E. (2010): Crops gone wild: evolution of weeds and invasives from domesticated ancestors. Evolutionary Applications. DOI:10.1111/j.1752-4571.2010.00140.x.

Ellstrand, N.C., Prentice, H.C., Hancock, J.F. (1999): Gene flow and introgression from domesticated plants into their wild relatives. Annual Review of Ecology, Evolution, and Systematics 30: 539-563.

Elmore, R.W., Roeth, F.W., Nelson, L.A., Shapiro, C.A., Klein, R.N., Knezevic, S.Z., Martin, A., (2001): Glyphosate-resistant soybean cultivar yields compared with sister lines. Agronomy Journal 93, 408-412.

Emberlin, J., Adams-Groom, B., Tidmarsh, J. (1999): The dispersal of maize (Zea mays) pollen. A report based on evidence available from publications and internet sites. A report commissioned by the Soil Association. National Pollen Research Unit, University College Worcester. Worcester, UK.

Engels H, Sinha A, Schuphan I, Eber S (2008): Small-scale dispersal of the European corn borer and its relevance for resistance management in Bt maize. J. Appl. Entomol. 132: 675-680.

EPA (2010): Cry1Ab and Cry1F Bacillus thuringiensis (Bt) Corn Plant-Incorporated Protectants. Biopesticides Registration Action Document.

Erasmus, A., Van Rensburg, J.B., Van den,Berg J. (2010): Effects of Bt maize on Agrotis segetum (Lepidoptera: Noctuidae): a pest of maize seedlings. Environmental Entomology 39 (2), 702-706. DOI:10.1603/EN09150.

Escher, N., Käch, B., Nentwig, W. (2000): Decomposition of transgenic Bacillus thuringiensis maize by microorganisms and woodlice Porcello scaber (Crustacea: Isopoda). Basic and Applied Ecology 1: 161-169.

European Commission (2002): Commission Decision (2002/623/EC) of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council of the deliberate release in to the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Off. J. Eur. Commun. L 200, 22-33.

European Commission (2003): Commission Directive 2003/63/EC amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use. http://www.emea.europa.eu/pdfs/human/pmf/2003-63-EC.pdf

European Commission (2010): A decade of EU-funded GMO research. Directorate-General for Research and Innovation.

European Community (1989): Council Directive 89/391/EEC of 12 June 1989 on the introduction of measures to encourage improvements in the safety and health of workers at work. OJ L 183, 29.6.1989, p. 1-8. http://eur-lex.europa.eu/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31989L0391&model=guichett

European Community (2001): Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Off. J. Eur. Commun. L 106, 1-39.

Evans, H.F. (2002): Environmental impact of Bt exudates from roots of genetically modified plants. In: UK Department for Environment, Food and Rural Affairs (Ed); DEFRA report (EPG 1/5/156).

Fakhrudin, B., Badariprasad, K.B., Krishnareddy, S., Prakash, H., Vijaykumar, B., Patil, V., Kuruvinashetti, M.S. (2003): Insecticide resistance in cotton bollworm, Helicoverpa armigera (Hubner) in South Indian cotton ecosystems. Resistant Pest Management Newsletter 12(2):13-16.

FAO (2008): Expert Consultation on Biosafety within a Biosecurity Framework: Contributing to Sustainable Agriculture and Food Production (28 February - 3 March 2006 FAO, Rome, Italy) – Final Report. In: Food and Agriculture Organization of the United Nations, Rome.

FAOSTAT (2005): Soybean Production Statistics. In: Food and Agriculture Organization of the United Nations, Rome.

Faria, C.A., Wäckers, F.L., Pritchard, J., Barrett, D.A., Turlings, T.C.J. (2007): High susceptibility of Bt maize to aphids enhances the performance of parasitoids of lepidopteran pests. Public Library of Science. DOI:10.1371/journal.pone.0000600.

Farinós, G.P., Poza, M. de la, Hernández-Crespo, P., Ortego, F., Castañera, P. (2004): Resistance monitoring of field populations of the corn borers Sesamia nonagrioides and Ostrinia nubilalis after 5 years of Bt maize cultivation in Spain. Entomologia Experimentalis et Applicata 110: 23-30.

Farinós, G.P., Poza, M. de la, Hernández-Crespo, P., Ortego, F., Castañera, P. (2008): Diversity and seasonal phenology of aboveground arthropods in conventional and transgenic maize crops in Central Spain. Biological Control 44: 362-371. DOI:10.1016/j.biocontrol.2007.11.007.

Farinós, G.P., Andreadis, S.S., Poza de la, M., Mironidis, K., Ortego, F., Savopoulou-Soultani, M., Castañera, P. (2011): Comparative assessment of the field-susceptibility of Sesamia nonagrioides to the Cry1Ab toxin in areas with different adoption rates of Bt maize and in Bt-free areas. Crop Protection, 1-5. DOI:10.1016/j.cropro.2011.03.011. TO.

Fedoroff, N.V. (2010): The past, present and future of crop genetic modification. New Biotechnology 27, 461-464. DOI:10.1016/j.nbt.2009.12.004.

Fedoroff, N.V., Battisti, D.S., Beachy, R.N., Cooper, P.J.M., Fischhoff, D.A., Hodges, C.N., Knauf, V.C., Lobell, D., Mazur, B.J., Molden, D., Reynolds, M.P., Ronald, P.C., Rosegrant, M.W., Sanchez, P.A., Vonshak, A., Zhu, J.-K. (2010): Radically Rethinking Agriculture for the 21st Century. Science 327, 833-834. DOI:10.1126/science.1186834.

Felke, M. and Langenbruch, G.A. (2005): Auswirkungen des Pollens von transgenem Bt-Mais auf ausgewählte Schmetterlingslarven. BfN-Skripten 157. http://www.bfn.de/fileadmin/MDB/documents/skript157.pdf (accessed 21 January, 2009)

Felke, M., Langenbruch, G.-A., Feiertag, S., Kassa, A. (2010): Effect of Bt-176 maize pollen on first instar larvae of the Peacock butterfly (Inachis io) (Lepidoptera; Nymphalidae): Environmental Biosafety Research 9 (1), 5-12. DOI:10.1051/ebr/2010006.

Felke, M., Lorenz, N., Langenbruch, G.A. (2002): Laboratory studies on the effects of pollen from Bt-maize on larvae of some butterfly species. Journal of Applied Entomology - Zeitschrift für Angewandte Entomologie 126: 320-325. DOI:10.1046/j.1439-0418.2002.00668.x.

Fénart, S., Austerlitz, F.C., Cuguen, J., Arnaud, J.F. (2007): Long distance pollen-mediated gene flow at a landscape level: the weed beet as a case study. Molecular Ecology 16: 3801-3813. DOI:10.1111/j.1365-294X.2007.03448.x.

Feng, P.C., Baley, G.J., Clinton, W.P., Bunkers, G.J., Murtaza, F.A., Paulitz, T.C., Kidwell, K.K. (2005): Glyphosate inhibits rust diseases in glyphosate-resistant wheat and soybean. Proceedings of the National Academy of Sciences of the USA 102: 17290-17295. DOI:10.1073/pnas.0508873102.

Fernandez-Cornejo, J., Caswell, M. (2006): The first decade of genetically engineered crops in the United States. In: USDA Economic Research Service (Eds); Economic Information Bulletin 1. http://www.ers.usda.gov/publications/eib11/eib11.pdf

Fernie, A.R., Tadmor, Y., Zamir, D. (2006): Natural genetic variation improving crop quality. Current Opin-ion in Plant Biology 9, 196-202.

Ferré, J., Van Rie, J. (2002): Biochemistry and genetics of insect resistance to Bacillus thuringiensis. Annual Review of Entomology 47: 501-533. DOI:10.1146/annurev.ento.47.091201.145234.

Ferré, J., Van Rie, J., MacIntosh, S.C. (2008): Insecticidal genetically modified crops and insect resistance management (IRM). In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), Integration of insect-restistant genetically modified crops within IPM programs, Springer Science + Business Media BV, pp. 41-85.

Ferry, N., Mulligan, E.A., Majerus, M.E.N., Gatehouse, A.M.R. (2007): Bitrophic and tritrophic effects of Bt Cry3A transgenic potato on beneficial, non-target, beetles. Transgenic Research 16: 795-812. DOI:10.1007/s11248-007-9088-9.

Filion, M. (2008): Do transgenic plants affect rhizobacteria populations? Microb. Biotechnol. 1: 463-475.

Finke, D.L. and Snyder, W.E. (2008): Niche partitioning increases resource exploitation by diverse communities. Science 321: 1488-1490. DOI:10.1126/science.1160854.

Firbank, L.G., Heard, M.S., Woiwod, I.P., Hawes, C., Haughton, A.J., Champion, G.T., Scott, R.J., Hill, M.O., Dewar, A.M., Squire, G.R., May, M.J., Brooks, D.R., Bohan, D.A., Daniels, R.E., Osborne, J.L., Roy, D.B., Black, H.I.J., Rothery, P., Perry, J.N. (2003a): An introduction to the farm scale evaluations of genetically modified herbicide-tolerant crops. Journal of Applied Ecology 40: 2-16.

Firbank, L.G., Perry, J.N., Squire, G.R., Bohan, D.A., Brooks, D.R., Champion, G.T., Clark, S.J., Daniels, R.E., Dewar, A.M., Haughton, A.J., Hawes, C., Heard, M.S., Hill, M.O., May, M.J., Osborne, J.L., Rothery, P., Roy, D.B., Scott, R.J., Woiwod, I.P. (2003b): The implications of spring-sown genetically modified herbicide-tolerant crops for farmland biodiversity: A commentary on the Farm Scale Evaluations of Spring Sown Crops. URL: http//www.defra.gov.uk/environment/gm/fse/results/fse-commentary.pdf

Firbank, L.G., Rothery, P., May, M.J., Clark, S.J., Scott, R.J., Stuart, R.C., Boffey, C.W.H., Brooks, D.R., Champion, G.T., Haughton, A.J., Hawes, C., Heard, M.S., Dewar, A.M., Perry, J.N., Squire, G.R. (2006): Effects of genetically modified herbicide-tolerant cropping systems on weed seedbanks in two years of following crops. Biology Letters 2: 140-143. DOI:10.1098/rsbl.2005.0390.

Fitt, G.P. (2008): Have Bt crops led to changes in insecticide use patterns and impacted IPM? In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), Integration of insect-restistant genetically modified crops within IPM programs, Springer Science + Business Media BV, pp. 303-328.

Fladung, M. (2011): Genetically modified trees for a sustainable, environmentally responsible and resource-saving production of wood for energy production. (original: Gentechnisch veranderte Baume fur eine nachhaltige, umweltvertragliche und ressourcenschonende Produktion von Holz fur die Energiegewinnung. Gesunde Pflanzen 63, 101-110.

Fladung, M., Altosaar, I., Bartsch, I., Baucher, M., Boscaleri, F., Gallardo, F., Häggman, H., Hoenicka, H. Nielsen, K., Paffetti, D., Séguin, A., Stotzky, G., Vettori, C. (2012): European discussion forum on transgenic tree biosafety. Nature Biotechnology 30, 37-38.

Fladung, M., Ewald, D. (2006): Tree Transgenesis – Recent Developments. Springer-Verlag, Berlin, Heidelberg, New York, 2006.

Flores, S., Saxena, D., Stotzky, G. (2005): Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biology & Biochemistry 37: 1073-1082. DOI:10.1016/j.soilbio.2004.11.006.

Foetzki, A., Winzeler, M., Boller, T., Felber, F., Gruissem, W., Keel, C., Keller, B., Mascher, F., Maurhofer, M., Nentwig, W., Romeis, J., Sautter, C., Schmid, B., Bigler, F. (2011): Field trials with genetically modified powdery mildew-resistant wheat. (original: Freilandversuche mit gentechnisch verandertem Weizen mit Mehltauresistenz.). Agrarforschung Schweiz , 18 , (10) 446-453.

Foolad, M.R. (2004): Recent advances in genetics of salt tolerance in tomato. Plant Cell, Tissue and Organ Culture 76: 101-119.

Ford, C.S., Allainguillaume, J., Grilli-Chantler, P., Cuccato, G., Allender, C.J., Wilkinson, M.J. (2006): Spontaneous gene flow from rapeseed (Brassica napus) to wild Brassica oleracea. Proceedings of the Royal Society, Series B 273: 3111-3115. DOI:10.1098/rspb.2006.3686.

Ford-Lloyd, B.V., Williams, J.T. (1975): A revision of Beta section Vulgares (Chenopodiaceae), with new light on the origin of cultivated beets. Botanical Journal of the Linnean Society 71: 89-102.

Fox, J.L. (2003): Resistance to Bt toxin surprisingly absent from pests. Nature 21: 958-959.

Franke, A.C., Breukers, M.L.H., Broer, W., Bunte, F., Dolstra, O., d'Engelbronner-Kolff, F.M., Lotz, L.A.P., Montfort van, J., Nokoloyuk, J., Rutten, M.M., Smulders, M.J.M., Wiel van de, C.C.M., Zijl van, M. (2011): Sustainability of current GM crop cultivation. Review of people, planet, profit effects of agricultural production of GM crops, based on the cases of soybean, maize, and cotton. Wageningen UR, Report 386.

Fredshavn, J.R., Poulsen, G., Huybrechts, I., Rüdelsheim, P. (1995): Competitiveness of transgenic oilseed rape. Transgenic Research, 4: 142-148.

Fu, G., Lees, R.S., Nimmo, D., Aw, D., Jin, L., Gray, P., Berendonk, T., White-Cooper, H., Scaife, S., Phuc, K.H., Marinotti, O., Jasinskiene, N., James, A., Alphey, L. (2010): Female-specific flightless phenotype for mosquito control. PNAS 107 (10), 4550–4554. DOI:10.1073/pnas.1000251107.

Fuchs, M., Chirco, E.M., McFerson, J.R., Gonsalves, D. (2004): Comparative fitness of a wild squash species and three generations of hybrids between wild × virus-resistant transgenic squash. Environ. Biosafety Res. 3, 17-28.

Gaines, T., Preston, C., Byrne, P., Henry, W.B., Westra, P. (2007): Adventitious Presence of Herbicide Resistant Wheat in Certified and Farm-Saved Seed Lots. Crop Science, 47, 751-756.

Gaines, T.A. , Zhang, W., Wang, D., Bukun, B., Chisholm, S.T., Shaner, D.L., Nissen, S.J., Patzoldt, W.L., Tranel, P.J., Culpepper, A.S., Grey, T.L., Webster, T.M., Vencill, W.K., Sammons, R.D., Jian, J., Preston, C., Leach, J.E., Westra, P. (2010): Gene amplification confers glyphosate resistance in Amaranthus palmeri. Proceedings of the National Academy of Sciences of the United States of America 107, 1029-1034.

Gallina, M.A. and Stephenson, G.R. (1992): Dissipation of [14C]Glufosinate ammonium in two Ontario soils. J. Agric. Food Chem. 40: 165-168.

Garcia, M., Ortego, F., Castanera, P., Farinos, G.P. (2010): Effects of exposure to the toxin Cry1Ab through Bt maize fed-prey on the performance and digestive physiology of the predatory rove beetle Atheta coriaria. Biological Control 55, 225-233. DOI:10.1016/j.biocontrol.2010.08.002.

Gardiner, M.A., Tuell, J.K., Isaacs, R., Gibbs, J., Ascher, J.S., Landis, D.A. (2010): Implications of Three Biofuel Crops for Beneficial Arthropods in Agricultural Landscapes. Bioenergy Research 3, 6-19 DOI:10.1007/s12155-009-9065-7.

Garnier, A., Deville, A., Lecomte, J. (2006): Stochastic modelling of feral plant populations with seed immigration and road verge management. Ecological Modelling, 197: 373-382.

Garnier, A. and Lecomte, J. (2006): Using spatial and stage-structured invasion model to assess the spread of feral population of transgenic oilseed rape. Ecological Modelling, 194: 141-149.

Garnier, A., Pivard, S., Lecomte, J. (2008): Measuring and modelling anthropogenic secondary seed dispersal along roadverges for feral oilseed rape. Basic and Applied Ecology, 9: 533-541.

Gašparič, M.B., Tengs, T., La Paz, J.L., Holst-Jensen, A., Pla, M., Esteve, T., Žel, J., Gruden, K. (2010): Comparison of nine different real-time PCR chemistries for qualitative and quantitative applications in GMO detection. Analytical and Bioanalytical Chemistry 396, 2023-2029. DOI: 10.1007/s00216-009-3418-0.

Gaspers, C., Siegfried, B.D., Spencer, T., Alves, A., Storer, N.P., Schuphan, I., Eber, S. (2010): Susceptibility of European and North American populations of the European corn borer to the Cry1F insecticidal prote. Baseline susceptibility of European corn borer. DOI:10.1111/j.1439-0418. 2010.01541.x.

Gassmann, A.J., Carrière, Y., Tabashnik, B.E. (2009): Fitness costs of insect resistance to Bacillus thuringiensis. Annu. Rev. Entomol. 54: 147-163.

Gassmann, A.J., Petzold-Maxwell, J.L., Keweshan, R.S., Dunbar, M.W. (2011): Fied-evolved resistance to Bt maize by Western corn rootworm. PLoS ONE 6, e22629.

Gathmann, A. and Bartsch, D. (2006): National coordination of GMO monitoring – a concept for Germany. Journal für Verbraucherschutz und Lebensmittelsicherheit (Journal of Consumer Protection and Food Safety), Volume 1, Supplement 1, p. 45-48.

Gathmann, A., Wirooks, L., Eckert, J., Schuphan, I. (2006a): Spatial distribution of Aglais urticae (L.) and its host plant Urtica dioica (L.) in an agricultural landscape: implications for Bt maize risk assessment and post-market monitoring. Environmental Biosafety Research 5: 27-36. DOI:10.1051/ebr:2006014.

Gathmann, A., Wirooks, L., Hothorn, L.A., Bartsch, D., Schuphan, I. (2006b): Impact of Bt-maize pollen (MON810) on lepidopteran larvae living on accompanying weeds. Molecular Ecology 15: 2677-2685. DOI: 10.1111/j.1365-294X.2006.02962.x.

Gay, P.B. and Gillespie, S.H. (2005): Antibiotic resistance markers in genetically modified plants: a risk to human health? The Lancet Infectious Diseases 5: 627-646.

Ge, X., d'Avignon, D.A., Ackerman, J.J.H., Sammons, R.D. (2010): Rapid vacuolar sequestration: the horseweed glyphosate resistance mechanism. Pest Management Science 66, 345-348. DOI:10.1002/ps.1911.

Gebhard, F., Smalla, K. (1998): Transformation of Acinobacter sp. Strain BD413 by transgenic sugar beet DNA. Applied and Environmental Microbiology 64: 1550-1554.

Gebhard, F. and Smalla, K. (1999): Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbiology Ecology 28: 261-272. DOI:10.1111/j.1574-6941.1999.tb00581.x.

Gianessi, L.P. (2005): Economic and herbicide use impacts of glyphosate-resistant crops. Pest Management Science, 61: 241-245.

Gianessi, L.P. (2008): Economic impacts of glyphosate-resistant crops. Pest Management Science, 64: 346-352.

Gianessi, L.P., Silvers, C.S., Sankula, S., Carpenter, J.E. (2002): Plant biotechnology: current and potential impact for improving pest management in US agriculture. An analysis of 40 case studies June 2002. Herbicide tolerant field corn. National Center for Food and Agricultural Policy, Washington, US. http://www.ncfap.org/documents/SweetCornHT.pdf

Gibbons, D.W., Bohan, D.A., Rothery, P., Stuart, R.C., Haughton, A.J., Scott, R.J., Wilson, J.D., Perry, J.N., Clark, S.J., Dawsson, R.J.G., Firbank, L.G. (2006): Weed seed resources for birds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. Proceedings of the Royal Society, Series B. DOI:10.1098/rspb.2006.3522.

Gimsing, A.L. and Borggard, O.K. (2002): Competitive adsorption and desorption of glyphosate and phosphate on clay silicates and oxides. Clay minerals, 37: 509-515.

Goldsborough, L.G., Brown, D.J. (1993): Dissipation of glyphosate and aminomethylphosphonic acid in water and sediments of boreal forest. Environmental Toxicology and Chemistry 12: 1139-1147. DOI:10.1897/1552-8618(1993)12[1139:DOGAAA]2.0.CO;2.

Gomez, I., Pardo-Lopez, L., Munoz-Garay, C., Fernandez, L.E., Perez, C., Sanchez, J., Soberón, M., Bravo, A. (2007): Role of receptor interaction in the mode of action of insecticidal Cry and Cyt toxins produced by Bacillus thuringiensis. Peptides 28: 169-173.

Gould, F.W. (1968): Grass systematics. McGraw Hill. New York, USA.

Gower, S.A., Loux, M.M., Cardina, J., Harrison, S.K. (2002): Effect of planting date, residual herbicide, and postemergence application timing on weed control and grain yield in glyphosate-tolerant corn (Zea mays). Weed Technology, 16: 448-494.

Gower, S.A., Loux, M.M., Cardina, J., Harrison, S.K., Sprankle, P.L., Probst, N.J., Bauman, T.T., Bugg, W., Curran, W.S., Currie, R.S., Harvey, R.G., Johnson, W.G., Kells, J.J., Owen, M.D.K., Regehr, D.L., Slack, C.H., Spaur, M., Sprague, C.L., VanGessel, M., Young, B.G. (2003): Effect of postemergence glyphosate application timing on weed control and grain yield in glyphosate-resistant corn: results of a 2-yr multistate study. Weed Technology, 17: 821-828.

Graef, F., Schütte, G., Winkel, B., Teichmann, H., Mertens, M. (2010): Scale Implications for Environmental Risk Assessment and Monitoring of the Cultivation of Genetically Modified Herbicide-Resistant Sugar Beet: A Review. Living Reviews in Landscape Research.

Gray, M.E., Sappington, T.W., Miller, N.J., Moeser, J., Bohn, M.O. (2009): Adaptation and invasiveness of Western corn rootworm: intensifying research on a worsening pest. Annual Review of Entomology 54, 303-321.

Greenpeace (2007): Gift im Gen-Mais. http://www.greenpeace.de/themen/gentechnik/gefahren_risiken/artikel/gift_im_gen_mais_report_zu_aktuellen_forschungsergebnissen/

Gressel, J. (Ed.) (2005): Crop ferality and volunteerism. CRC Press. Boca Raton.

Gressel, J. (2010): Needs for and environmental risks from transgenic crops in the developing world. New Biotechnology 27, 522-527. DOI:10.1016/j.nbt.2010.05.015.

Grichar, W.J. and Minton, B.W. (2006): Supplementary weed control using soil-applied herbicides in glyphosate-resistant maize in Texas. Crop Protection, 25: 1071-1074.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Cortet, J., Andersen, M.N., Krogh, P.H. (2007a): Microbial and microfaunal community structure in cropping systems with genetically modified plants. Pedobiologia 51: 195-206. DOI:10.1016/j.pedobi.2007.04.002.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimgeour, C., Andersen, M.N., Cortet, J., Messéan, A., Sausse, C., Lacroix, B., Krogh, P.H. (2005): A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the Bacillus thuringiensis CryIAb toxin. Plant and Soil 275: 135-146. DOI:10.1007/s11104-005-1093-2.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimgeour, C., Cortet, J., Foggo, A., Hackett, C.A., Krogh, P.H. (2006): Soil microbial and faunal community responses to Bt maize and insecticide in two soils. Journal of Environmental Quality 35: 734-741. DOI:10.2134/jeq2005.0344.

Griffiths, B.S., Heckmann, L.H., Caul, S., Thompson, J., Scrimgeour, C., Krogh, P.H. (2007b): Varietal effects of eight paired lines of transgenic Bt maize and near-isogenic non-Bt maize on soil microbial and nematode community structure. Plant Biotechnology Journal 5: 60-68. DOI: 10.1111/j.1467-7652.2006.00215.x.

Gruber, H., Paul, V., Meyer, H.D.H., Müller, M. (2011a): Determination of insecticidal Cry1Ab protein in soil collected in the final growing seasons of nine-year field trial of Bt-maize MON810. Transgenic Research. DOI:10.1007/s11248-011-9509-7.

Gruber, H., Paul, V., Guertler, P., Spiekers, H., Tichopad, A., Meyer, H.H.D., Müller, M. (2011b): Fate of Cry1Ab Protein in Agricultural Systems under Slurry Management of Cows Fed Genetically Modified Maize (Zea mays L.) MON810: A Quantitative Assessment. Journal of Agricultural and Food Chemistry, DOI:101021/jf200854n.

Guéritaine, G., Sester, M., Eber, F., Chèvre, A.M., Darmency, H. (2002): Fitness of backcross six of hybrids between transgenic oilseed rape (Brassica napus) and wild radish (Raphanus raphanistrum). Molecular Ecology 11: 1419-1426. DOI:10.1046/j.1365-294X.2002.01540.x.

Gunning, R.V., Dang, H.T., Kemp, F.C., Nicholson, I.C., Moores, G.D. (2005): New resistance mechanism in Helicoverpa armigera threatens transgenic crops expressing Bacillus thuringiensis Cry1Ac toxin. Applied and Environmental Microbiology 71: 2558-2563. DOI:10.1128/AEM.71.5.2558-2563.2005.

Habuštová, O., Doležal, P., Hussein, H.M., Spitzer, L., Turanli, F., Růžička, V., Sehnal, F. (2007): Lack of effect of maize expressing bacterial toxin Cry1Ab on the composition of insect communities 3rd EIGMO Meeting; Ecological Impact of Genetically Modified Organisms (EIGMO), 23-25 May 2007, Warsaw, Poland: 40.

Habuštová, O., Turanli, F., Doležal, P., Růžička, V., Spitzer, L., Hussein, H.M (2006): Environmental impact of Bt maize – three year experience. IOBC wprs Bulletin 29: 57-63.

Hails, R.S., Raymond, B. (2004): Insect-resistant transgenic plants and their environmental impact. In: Nijs, H.C.M. den, Bartsch, D., Sweet, J. (Eds); Introgression from genetically modified plants into wild relatives: 279-295. CABI Publishing.

Hails, R.S., Morley, K. (2005): Genes invading new populations: a risk assessment perspective. Trends in Ecology and Evolution 20: 242-252. DOI:10.1016/j.tree.2005.02.006.

Halfhill, M.D, Sutherland, J.P., Moon, H.S., Poppy, G.M., Warwick, S.I., Weissinger, A.K., Rufty, T.W., Raymer, P.L., Steward, N.C. (2005): Growth, productivity, and competiveness of introgressed weedy Brassica rapa hybrids selected for the presence of Bt cry1Ac and GFP transgenes. Molecular Ecology 14: 3177-3189.

Halfhill, M.D, Zhu, B., Warwick, S.I., Raymer, P.L., Millwood, R.J., Weissinger, A.K., Stewart Jr., C.N. (2004): Hybridization and backcrossing between transgenic oilseed rape and two related weed species under field conditions. Environmental Biosafety Research 3: 73-81. DOI:10.1051/ebr:2004007.

Hall, L.M, Rahman, M.H., Gulden, R.H., Thomas, A.G. (2005): Volunteer oilseed rape – will herbicide-resistence traits assist ferality? In: Gressel, J. (Ed); Crop ferality and volunteerism: 59-79. CRC Press.

Halpin, C., Thain, S.C., Tilston, E.L., Guiney, E., Lapierre, C., Hopkins, D.W. (2007): Ecological impacts of trees with modified lignin. Tree Genetics & Genomes 3: 101-110. DOI:10.1007/s11295-006-0060-2.

Han, P., Niu, C.-Y., Lei, C.-L., Cui, J.-J., Desneux, N. (2010): Quantification of toxins in a Cry1Ac + CpTI cotton cultivar and its potential effects on the honey bee Apis mellifera L.. Ecotoxicology (2010) 19, 1452-1459. DOI:101007/s10646-010-0530-z.

Haney, R.L., Senseman, S.A., Hons, F.M. (2002): Effect of Roundup Ultra on microbial activity and biomass from selected soils. Journal of Environmental Quality 31: 730-735.

Haney, R.L., Senseman, S.A., Hons, F.M., Zuberer, D.A. (2000): Effect of glyphosate on microbial activity and biomass. Weed Science Journal 48: 89-93.

Hannula, S.E., Boer de, W., Veen van, J.A. (2010): In situ dynamics of soil fungal communities under different genotypes of potato including a genetically modified cultivar. Soil Biology & Biochemistry 42, 2211-2223. DOI:10.1016/j.soilbio.2010.08.020.

Harfouche et al. (2011): Tree genetic engineering and applications to sustainable forestry and biomass production, Trends in Biotechnology 29, 9-17.

Harfouche et al. (2012): Accelerating the domestication of forest trees in a changing world, Trends in Plant Science 17 p. 64-72.

Harrigan, G.G., Lundry, D., Drury, S., Berman, K., Riordan, S.G., Nemeth, M.A., Ridley, W.P., Glenn, K.C. (2010): Natural variation in crop composition and the impact of transgenesis. Nature Biotechnology 28, 402-404.

Harwood, J.D. and Obrycki, J.J. (2006): The detection and decay of Cry1Ab Bt-endotoxins within non-target slugs, Deroceras reticulatum (Mollusca: Pulmonata), following consumption of transgenic corn. Biocontrol Science and Technology 16: 77-88. DOI:10.1080/09583150500257216.

Harwood J,D., Samson, R.A., Obrycki, J.J. (2007): Temporal detection of Cry1Ab-endotoxins in coccinellid predators from fields of Bacillus thuringiensis. Bull. Entomol. Res. 97: 643-648.

Harwood, J.D., Wallin, W.G., Obrycki, J.J. (2005): Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Molecular Ecology 14: 2815-2823. DOI:10.1111/j.1365-294X.2005.02611.x.

Hauser, T.P., Bjørn, G.K., Magnussen, L., Shim, S.I. (2004): Hybrids between cultivated and wild carrots: a life history. In: Den Nijs HCM, Bartsch D, Sweet J (eds). Introgression from Genetically Modified Plants into Wild Relatives. CABI Publishing: Wallingford. pp 41-51.

Hauser, T.P., Jørgensen, R.B., Østergard, H. (1998b): Fitness of backcross and F2 hybrids between weedy Brassica rapa and oilseed rape (Brassica napus). Heredity 81: 436-443.

Hauser, T.P., Shaw, R.G., Østergard, H. (1998a): Fitness of F1 hybrids between weedy Brassica rapa and oilseed rape. Heredity 81: 429-435.

Hawes, C., Haughton, A.J., Osborne, J.L., Roy, D.B., Clark, S.J., Perry, J.N., Rothery, P., Bohan, D.A., Brooks, D.R, Champion, G.T., Dewar, A.M., Heard, M.S., Woiwod, I.P., Daniels, R.E., Young, M.W., Parish, A.M., Scott, R.J., Firbank, L.G., Squire, G.R. (2003): Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Philosophical Transactions of the Royal Society, B 358: 1899-1913. DOI:10.1098/rstb.2003.1406.

Hawes, C., Squire, G.R., Hallett, P.D., Watson, C.A., Young, M. (2010): Arable plant communities as indicators of farming practice. Agriculture, Ecosystems and Environment, 1-10. DOI:10.1016/ j.agee.2010.03.010.

Hayes, K.R., Gregg, P.C., Gupta, V.V.S.R., Jessop, R., Lonsdale, W.M., Sindel, B., Stanley, J., Williams, C.K. (2004): Identifying hazards in complex ecological systems. Part 3: Hierarchical holographic model for herbicide tolerant oilseed rape. Environmental Biosafety Research 3: 109-128. DOI:10.1051/ebr:2004012.

Head, G., Brown, C.R., Groth, M.E., Duan, J.J. (2001): Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomol. Exp. Appl. 99: 37-45.

Heap, I. (2011): The international survey of herbicide resistant weeds. www.weedscience.com

Heard, M.S., Hawes, C., Champion, G.T., Clark, S.J., Firbank, L.G., Haughton, A.J., Parish, A.M., Perry, J.N., Rothery, P., Scott, R.J., Skellern, M.P., Squire, G.R., Hill, M.O. (2003a): Weeds in field with contrasting conventional and genetically modified herbicide-tolerant crops. I. Effects on abundance and diversity. Philosophical Transactions of the Royal Society, B 358: 1819-1832. DOI:10.1098/rstb.2003.1402.

Heard, M.S., Hawes, C., Champion, G.T., Clark, S.J., Firbank, L.G., Haughton, A.J., Parish, A.M., Perry, J.N., Rothery, P., Roy, D.B., Scott, R.J., Skellern, M.P., Squire, G.R., Hill, M.O. (2003b): Weeds in field with contrasting conventional and genetically modified herbicide-tolerant crops. II. Effects on individual species. Philosophical Transactions of the Royal Society, B 358: 1833-1846. DOI:10.1098/rstb.2003.1401.

Heard, M.S., Rothery, P., Perry, J.N., Firbank, L.G. (2005): Predicting longer-term changes in weed populations under GMHT crop management. Weed Research 45: 331-338. DOI:10.1111/j.1365-3180.2005.00465.x.

Heckmann, L.H., Griffiths, B.S., Caul, S., Thompson, J., Pusztai-Carey, M., Moar, W.J., Andersen, M.N., Krogh, P.H. (2006): Consequences for Protaphorura armata (Collembola: Onychiuridae) following exposure to genetically modified Bacillus thuringiensis (Bt) maize and non-Bt maize. Environmental Pollution 142: 212-216. DOI:10.1016/j.envpol.2005.10.008.

Heerwaarden Van, J., Eeuwijk van, F.A., Ross-Ibarra, J. (2010): Genetic diversity in a crop meta population, Heredity (104), 28-39.

Heink, U., Bartz, R., Kowarik, I. (2010): How Useful are the Concepts of Familiarity, Biological Integrity, and Ecosystem Health for Evaluating Damages by GM Crops? Journal of Agricultural Environmental Ethics. DOI:10.1007/s10806-010-9289-8.

Hellmich, R.L., Siegfried, B.D., Sears, M.K., Stanley-Horn, D.E., Daniels, M.J., Mattila, H.R., Spencer, T., Bidne, K.G., Lewis, L.C. (2001): Monarch larvae sensitivity to Bacillus thuringiensis-purified proteins and pollen. Proceedings of the National Academy of Sciences of the USA 98: 11925-11930. DOI:10.1073/pnas.211297698.

Hendrickx, F., Maelfait, J.P., Wingerden, W. van, Schweiger, O., Speelmans, M., Aviron, S., Augenstein, I., Billeter, R., Bailey, D., Bukacek, R., Burel, F., Diekötter, T., Dirksen, J., Herzog, F., Liira, J., Roubaliva, M., Vandomme, V., Bugter, R. (2007): How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. Journal of Applied Ecology 44: 340-351. DOI:10.1111/j.1365-2664.2006.01270.x.

Henry, C., Morgan, D., Weekes, R., Daniels, R., Boffey, C. (2003): Farm scale evaluations of GM crops: monitoring gene flow from GM crops to non-GM equivalent crops in the vicinity (contract reference EPG 1/5/138). Part I: Forage Maize. DEFRA, CEH, CSL.

Herman, R.A. (2010): Ecological risk assessment for transgenic crops: separating the seed from the chaff. Trends in Biotechnology. DOI:10.1016/j.tibtech.2010.01.005.

Herman, R.A., Song, P., Zhuang, M. (2011): Safety risks of cryptic reading frames and gene disruption due crop transgenesis. What are the odds? Landes Bioscience 2 (1), 4-6. DOI:10.4161/gmcr.2.1. 15622.

Hernandez, A., Garcia-Plazaola, J.I., Becerril, J.M. (1999): Glyphosate effects on phenolic metabolism of nodulated soybean (Glycine max L. Merr.). J. Agric. Food Chem. 47: 2920-2925. DOI:10.1021/ jf981052z.

Heuberger, S., Ellers-Krik, C., Tabashnik, B.E., Carriere, Y. (2010): Pollen- and Seed-Mediated Transgene Flow in Commercial Cotton Seed Production Fields. Plos One 5(11). DOI:10.1371/ journal.pone.0014128.

Heuer, H. and Smalla, K. (2007): Horizontal gene transfer between bacteria. Environmental Biosafety Research 6: 3-13. DOI:10.1051/ebr:2007034.

Hibbard, B.E., Meihls, L.N., Ellersieck, M.R., Onstad, D.W. (2010): Density-Dependent and Density-Independent Mortality of the Western Corn Rootworm: Impact on Dose Calculations of Rootworm-Resistant Bt Corn. Journal of Economic Entomology 103 (1), 77-84. DOI:10.1603/EC09277.

Hilbeck, A., Baumgartner, M., Fried, P.M., Bigler, F. (1998b): Effects of transgenic Bacillus thuringiensis corn-fed prey on mortality and development time of immature Chrysoperla carnea (Neuroptera: Chrysopidae). Environmental Entomology 27: 480-487.

Hilbeck, A., McMillan, J.M., Meier, M., Humbel, A., Schlaepfer-Miller, J., Trtikova M. (2012b): A controversy re-visited: Is the coccinellid Adalia bipunctata adversely affected by Bt toxins? Environmental Sciences Europe 2012, 24:10.

Hilbeck, A., Meier, M.S., Raps, A. (2000): Review on non-target organisms and Bt-plants. Report to Greenpeace International by EcoStrat-GmbH-Ecological Technology Assessment & Environmental Consulting. Zürich.

Hilbeck, A., Meier, M., Römbke, J., Jänsch, S., Teichmann, H., Tappeser, B. (2011): Environmental risk assessment of genetically modified plants – concepts and controversies. Environmental Sciences Europe 23 (13). DOI:10.1186/2190-4715-23-13.

Hilbeck, A. Meier, M., Trtikova, M. (2012a): Underlying reasons of the controversy over adverse effects of Bt toxins on lady beetle and lacewing larvae. Environmental Sciences Europe 24:9 DOI:10.1186/2190-4715-24-9.

Hilbeck, A., Moar, W.J., Pusztai-Carey, M., Filippini, A., Bigler, F. (1998a): Toxicity of Bacillus thuringiensis CryIAb toxin to the predator Chrysoperla carnea (Neuroptera: Chrysopidae) using diet incorporated bioassays. Environmental Entomology 27: 1255-1263.

Hilbeck, A., Moar, W.J., Pusztai-Carey, M., Filippini, A., Bigler, F. (1999): Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator Chrysoperla carnea. Entomol. Exp. Appl. 91: 305-316.

Hilbeck, A. and Schmidt, J.E.U. (2006): Another view on Bt proteins – How specific are they and what else might they do? Biopesticides International 2: 1-50.

Himanen, S.J., Nerg, A.M., Poppy, G., Steward, C.N., Holopainen, J.K. (2010): Abiotic stress and transgenics: Implications for reproductive success and crop-to-wild gene flow in Brassicas. Basic and Applied Ecology. DOI:10.1016/j.baae.2010.06.007.

Hjältén, J., Lindau, A., Wennström, A., Blomberg, P., Witsell, J., Hurry, W. (2007): Unintentional changes of defense traits in GM trees can affect plant-herbivore interactions. Basic and Applied Ecology 8: 434-443.

Hjältén, J.H., Lindau, A., Wennström, A., Blomberg, P., Witzell, J., Hurry, V., Ericson, L., Moritz, T., Karlsson, J. (2008): Vole response to unintentional changes in the chemistry of GM poplars. Chemoecology 18: 227-231.

Hofmann, F., Epp, R., Kalchschmid, A., Kratz, W., Kruse, L., Kuhn, U., Maisch, B., Müller, E., Ober, S., Radtke, J., Schlechtriemen, U., Schmidt, G., Schröder, W., von der Ohe, W., Vögel, R., Wedl, N., Wosniok, W. (2010): Monitoring of Bt-Maize pollen exposure in the vicinity of the nature reserve Ruhlsdorfer Bruch in northeast Germany 2007 to 2008. Umweltwissenschaften und Schadstoff-Forschung 22, 229-251.

Hofmann, F., Otto, M., Kuhn, U., Ober, S., Schlechtriemen, U., Vögel, R. (2011): A New Method for in Situ Measurement of Bt-Maize Pollen Deposition Host-Plant Leaves. Insects 2, 12-21. DOI: 10.3390/insects2010012.

Hoheisel, G.A. and Fleischer, S.J. (2007): Coccinellids, aphids, and pollen in diversified vegetable fields with transgenic and isoline cultivars. Journal of Insect Science 7. www.insectscience.org/7.61

Hönemann, L. and Nentwig, W. (2009): Are survival and reproduction of Enchytraeus albidus (Annelida: Enchytraeidae) at risk by feeding on Bt-maize litter? European Journal of Soil Biology, pre-print published online, DOI:10.1016/j.ejsobi.2009.03.001.

Hönemann, L., Nentwig, W. (2010): Does feeding on Bt-maize affect the slug Arion vulgaris (Mollusca: Arionidae)? Biocontrol Science and Technology, Vol. 20, No. 1, 13-18; DOI:10.1080/09583150903308707.

Hönemann, L., Zurbrügg, C., Nentwig, W. (2008): Effects of Bt-corn decomposition on the composition of soil meso- and macrofauna. Applied Soil Ecology, 40: 203-209.

Hönicka, H. and Fladung, M. (2006a): Genome instability in woody plants derived from genetic engineering. In: Tree Transgenesis: Recent developments Fladung, M., Ewald, D. (Eds.). Berlin: Springer, 301-321.

Hönicka, H. and Fladung, M. (2006b): Biosafety in Populus spp. and other forest trees: from non-native species to taxa derived from traditional breeding and genetic engineering. Trees 20, 131-144.

Hönicka, H., Nowitzki, O., Hanelt, D., Fladung, M. (2008): Heterologous overexpression of the birch FRUITFULL-like MADS-box gene BpMADS4 prevents normal senescence and winter dormancy in Populus tremula L. Planta 227, 1001-1011.

Hooftmann, D.A.P., Flavell, A.J., Jansen, H., Nijs den, H.C.M., Syed, N.H., Sørensen, A.P., Orozco-ter Wengel, P., Wiel van de, C.M. (2011): Locus-dependent selection in crop-wild hybrids of lettuce under field conditions and its implication for GM crop development. Evolutionary Applications. DOI: 10.1111/j.1752-4571.2011.00188.x.

Hopkins, D.W. and Gregorich, E.G. (2003): Detection and decay of the Bt endotoxin in soil from a field trial with genetically modified maize. European Journal of Soil Science 54: 793-800. DOI: 10.1046/j.1365-2389.2003.00563.x.

Hopkins, D.W. and Gregorich, E.G. (2005): Decomposition of residues and loss of the δ-endotoxin from transgenic (Bt) corn (Zea mays L.) in soil. Canadian Journal of Soil Science 85: 19-26.

Höss, S., Nguyen, H.T., Menzel, R., Pagel-Wieder, S., Miethling-Graf, R., Tebbe, C.C., Jehle, J.A., Traunspurger, W. (2011): Assessing the risk posed to free-living soil nematodes by a genetically modified maize expressing the insecticidal Cry3Bb1 protein. Science of the Total Environment 409, 2674-2684. DOI:10.1016/j.scitotenv.2011.03.041.

Huang, F., Andow, D.A., Buschmann, L.L. (2011): Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America. Entomologia Experimentalis et Applicata 140, 1-16. DOI:10.1111/j.1570-7458.2011.01138.x.

Huang, F., Buschman, L.L., Higgins, R.A., Li, H. (2002): Survival of Kansas Dipel-resistant European corn borer (Lepidoptera: Crambidae) on Bt and non Bt corn hybrids. J. Econ. Entomol. 95: 614-621.

Huang, F., Ghimire, M.N., Leonard, B.R., Wang, J., Daves, C., Levy, R., Cook, D., Head, G.P., Yang, Y., Temple, J., Gerguson, R. (2011): F2 screening for resistance to pyramided Bacillus thuringiensis maize in Louisiana and Mississippi populations of Diatraea saccharalis (Lepidoptera: Crambidae): Pest Management Science. DOI:10.1002/ps.2182.

Huang, Z.Y., Hanley, A.V., Pett, W.L., Langenberger, M., Duan, J.J. (2004): Field and semifield evaluation of impacts of transgenic canola pollen on survival and development of worker honey bees. Journal of Economic Entomology 97: 1517-1523.

Huang, F., Higgins, R.A., Buschman, L.L. (1997): Baseline susceptibility and changes in susceptibility to Bacillus thuringiensis subsp. kurstaki under selection pressure in European corn borer (Lepidoptera: Pyralidae). Journal of Economic Entomology 90: 1137-1143.

Huang, J., Hirji, R., Adam, L., Rozwadowski, K.L., Hammerlindl, J.K., Keller, W.A., Selvaraj, G. (2000): Genetic engineering of glycinebetaine production toward enhancing stress solerance in plants: Metabolic limitations. Plant Physiology 122: 747-756.

Huang, F., Leonard, B.R., Cook, D.R., Lee, D.R., Andow, D.A., Baldwin, J.L., Tindall K.V., Wu, X. (2007): Frequency of alleles conferring resistance to Bacillus thuringiensis maize in Louisiana populations of the southwestern corn borer. Entomologia Experimentalis et Applicata 122: 53-58.

Hunt, T.E., Higley, L.G., Witkowski, J.F., Young, L.J., Hellmich, R.L. (2001): Dispersal of adult European corn borer (Lepidoptera: Crambidae) within and proximal to irrigated and non-irrigated corn. J. Econ. Entomol. 94: 1369-1377.

Hunter, P. (2011): A new era for plant biotechnology. EMBO reports 12 (6), 504-507. DOI: 10.1038/embor.2011.91.

Hüsken, A., Prescher, S., Schienmann, J. (2010): Evaluating biological containment strategies for pollen-mediated gene flow. Environmental Biosafety Research. DOI:10.1051/ebr/2010009.

Hutchison, W.D., Burkness, E.C., Mitchell, P.D., Moon, R.D., Leslie, T.W., Fleischer, S.J., Abrahamson, M., Hamilton, K.L., Steffey, K.L., † Gray, M.E., Hellmich, R.L., Kaster, L.V., Hunt, T.E., Wright, R.J., Pecinovsky, K., Rabaey, T.L., Flood, B.R., Raun, E.S. (2010): Areawide Suppression of European Corn Borer with Bt Maize Reaps Savings to Non-Bt Maize Growers. Science Vol. 330.

Hutchison, W.D., Hunt, T.E., Hein, G.L., Steffey, K.L., Pilcher, C.D., Rice, M.E. (2011): Genetically engineered Bt corn and range expansion of the Western Bean Cutworm (Lepidoptera: Noctuidae) in the United States: A Response to Greenpeace Germany J. Integ. Pest Mngmt. 2(3): 2011;DOI:http://dx.doi.org/10.1603/IPM11016

Icoz, I., Saxena, D., Andow, D.A., Zwahlen, C., Stotzky, G. (2008): Microbial populations and enzyme activities in soil in situ under transgenic corn expressing cry proteins from Bacillus thuringiensis. Journal of Environmental Quality 37: 647-662.

Icoz, I. and Stotzky, G. (2007): Cry3Bb1 protein from Bacillus thuringiensis in root exudates and biomass of transgenic corn does not persist in soil. Transgenic Research. DOI:10.1007/s11248-007-9133-8.

Icoz, I. and Stotzky, G. (2008): Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology & Biochemistry 40: 559-586. DOI:10.1016/j.soilbio.2007.11.002.

Ives, A.R. and Andow, D.A. (2002): Evolution of resistance to Bt crops: directional selection in structured environments. Ecol. Lett. 5: 792-801

Jaggard, K.W., Qi, A., Ober, E.S. (2010): Possible changes to arable crop yields by 2050. Philosophical Transaction of the Royal Society B 365, 2835-2851. DOI:10.1098/rstb.2010.0153.

Jasinski, J.R., Eisley, J.B., Young, C.E., Kovach, J., Willson, H. (2003): Select nontarget arthropod abundance in transgenic and nontransgenic field crops in Ohio. Environmental Entomology 32: 407-413.

Jensen, P.D., Dively, G.P., Swan, C.M., Lamp, W.O. (2010): Exposure and Nontarget Effects of Transgenic Bt Corn Debris in Streams. Environmental Entomology 39 (2), 707-714. DOI: 10.1603/EN09037.

Jiang, Z., Xia, H., Basso, B., Lu, B.R. (2011): Introgression from cultivated rice influences genetic differentiation of weedy rice populations at a local spatial scale. Theoretical and Applied Genetics.

Jiménez-Juárez, A., Muñoz-Garay, C., Gómez, I., Saab-Rincon, G., Damian-Alamazo, J.Y., Gill, S.S., Soberón, M., Bravo, A. (2007): Bacillus thuringiensis Cry1Ab mutants affecting oligomer formation are non-toxic to Manduca sexta larvae. Journal of Biological Chemistry, 282: 21222-21229.

Joensen, L., Semino, S., Paul, H. (2005): Argentina: A case study on the impact of genetically engineered soya. A report prepared for the Gaia Foundation. The GAIA Foundation. London, UK.

Johnson, K.L., Raybould, A. F., Hudson, M. D., Poppy, G. M. (2007): How does scientific risk assessment of GM crops fit within the wider risk analysis? Trends Plant Sci., 12 (1), 1-5.

Johnson, W.G., Davis, V.M., Kruger, G.R., Weller, S.C. (2009): Influence of glyphosate-resistant cropping systems on weed species shifts and glyphosate-resistant weed populations. European Journal of Agronomy 31, 162-172.

Jørgensen, R.B. (2007): Oilseed rape: Co-existence and gene flow from wild species. In: Rapeseed Breeding, Volume 45, Advances in Botanical Research (ed. Surinder Gupta). Elsevier.

Jørgensen, R.B., Andersen, B. (1994): Spontaneous hybridization between oilseed rape (Brassica napus) and weedy Brassica campestris (=rapa) – a risk of growing genetically-modified oilseed rape. American Journal of Botany 81: 1620-1626.

Jung, H.G. and Sheaffer, C.C. (2004): Influence of Bt transgenes on cell wall lignification and digestibility of maize stover for silage. Crop Science 44: 1781-1789.

Jurat-Fuentes, J.L., Gould, F., Adang, M.J. (2003): Dual resistance to Bacillus thuringiensis Cry1Ac and Cry2Aa toxins in Heliothis virescens suggests multiple mechanisms of resistance. Applied and Environmental Microbiology 69: 5898-5906. DOI:10.1128/AEM.69.10.5898-5906.200.

Kaldorf, M., Fladung, M., Muhs, H.-J., Buscot, F. (2002): Mycorrhizal colonization of transgenic aspen in a field trial. Planta 214: 653-660.

Kaphengst; T., El Benni, N., Evans, C., Finger, R., Herbert, S., Maorse, S., Stupak, N. (2011): Assessment of the economic performance of GM crops worldwide. Eidgenössische Technische Hochschule Zürich, ENV.B.3/ETU/2009/0010.

Karim, S., Aronsson, H., Ericson, H., Pirhonen, M., Leyman, B., Welin, B., Mäntylä, E., Palva, E.T., Dijck, P. van, Holmström, K.O. (2007): Improved drought tolerance without undesired side effects in transgenic plants producing trehalose. Plant Molecular Biology 64: 371-386. DOI:10.1007/s11103-007-9159-6.

Kay, E., Vogel, T.M., Bertolla, F., Nalin, R., Simonet, P. (2002): In situ transfer of antibiotic resistance genes from transgenic (transplastomic) tobacco plants to bacteria. Applied and Environmental Microbiology 68: 3345-3351.

Keese, P. (2008): Risks from GMOs due to horizontal gene transfer. Environmental Biosafety Research, 7: 123-149.

Kennedy, G.G. (2008): Integration of insect-resistant genetically modified crops within IPM programs. In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), Integration of insect-restistant genetically modified crops within IPM programs, Springer Science + Business Media BV, pp. 1-26.

King, C.A., Purcell, L.C., Vories, E.D. (2001): Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to foliar glyphosate applications. Agronomy Journal 93: 179-186.

Kjaer, C., Damgaard, C., Lauritzen, A.J. (2010): Assessment of effects of Bt-oilseed rape on large white butterfly (Pieris brassicae) in natural habitats. Entomologia Experimentalis et Applicata 134, 304-311. DOI:10.1111/j.1570-7458.2009.00958.x.

Kleter, G.A., Bhula, R., Bodnaruk, K., Carazo, E., Felsot, A.S., Harris, C.A., Katayama, A., Kuiper, H.A., Racke, K.D., Rubin, B., Shevah, Y., Stephenson, G.R., Tanaka, K., Unsworth, J., Wauchope, R.D., Wong, S.S. (2007): Altered pesticide use on transgenic crops and the associated general impact from an environmental perspective. Pest Management Science 63: 1107-1115. DOI:10.1002/ps.1448.

Kleter, G.A., Harris, C., Stephenson, G., Unsworth, J. (2008): Comparison of herbicide regimes and the associated potential environmental effects of glyphosate-resistant crops versus what they replace in Europe. Pest Management Science 64: 479-488.

Klinger, T., Ellstrand, N.C. (1994): Engineered genes in wild populations: fitness of weed-crop hybrids of Raphanus sativus. Ecological Applications 4: 117-120. DOI:10.2307/1942121.

Knecht, S., Romeis, J., Malone, L.A., Candolfi, M.P., Garcia-Alonso, M., Habustova, O., Huesing, J.E., Kiss, J., Nentwig, W., Pons, X., Rauschen, S., Szénási, Á., Bigler, F. (2010): A faunistic database as a tool for identification and selection of potential non-target arthropod species for regulatory risk assessment of GM maize. IOBC/wprs Bulletin Vol. 52, 65-69.

Knispel, A.L., McLachlan, S.M., Acker, R.C. van, Friesen, L.F. (2008): Gene flow and multiple herbicide resistance in escaped canola populations. Weed Science Journal 56: 72-80. Kogel, K.H., Voll, L.M., Schafer, P., Jansen, C., Wu, Y.C., Langen, G., Imani, J., Hofmann, J., Schmiedl, A., Sonnewald, S., von Wettstein, D., Cook, R.J., Sonnewald, U. (2010): Transcriptome and metabolome profiling of field-grown transgenic barley lack induced differences but show cultivar-specific variances. PNAS 107 (14), 6198–6203; DOI:10.1073/pnas.1001945107.

Koger, C.H., Poston, D.H., Hayes, H.M., Montgomery, R.E. (2004): Glyphosate-resistant horseweed (Conyza canadensis) in Mississippi. Weed Technology 18: 820-825.

Koziel, H.G., Beland, G.L., Bowman, C., Carozzi, N.B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K., Maddox, D., McPherson, K., Meghji, M.R., Merlin, E., Rhodes, R., Warren, G.W., Wright, M., Evola, S.V. (1993): Field performance of elite transgenic maize plants expressing an insecticidal protein derived from Bacillus thuringiensis. Bio/Technology 11: 194-200. DOI:10.1038/nbt0293-194.

Koziolek, C. (2009): Biosafety assessment and benefits for co-existence of biological contained plants-regulatory assessment in the EU-project Transcontainer. Journal für Verbraucherschutz und Lebensmittelsicherheit Volume 3, Supplement 2, p. 41.

Kramarz, P.E., Vaufleury, A. de, Carey, M. (2007a): Studying the effect of exposure of the snail Helix aspersa to the purified Bt toxin, Cry1Ab. Applied Soil Ecology 37: 169-172. DOI: 10.1016/j.apsoil.2007.06.006.

Kramarz, P.E., Vaufleury, A. de, Zygmunt, P.M.S., Verdun, C. (2007b): Increased response to cadmium and Bacillus thuringiensis maize toxicity in the snail Helix aspersa infected by the nematode Phasmarhabditis hermaphrodita. Environmental Toxicology and Chemistry 26: 73-79. DOI: 10.1897/06-095R.1.

Krausz, R.F. and Young, B.G. (2001): Response of glyphosate-resistant soybean (*Glycine max)* to trimethylsulfonium and isopropylamine salts of glyphosate. Weed Technology 15, 745-749.

Krebs, J.R., Wilson, J.D., Bradbury, R.D., Sirwardena, G.M. (1999): The second Silent Spring? Nature, 400: 611-612.

Kremer, R.J., Means, N.E., Kim, S. (2005): Glyphosate affects soybean root exudation and rhizosphere micro-organisms. International Journal of Environmental Analytical Chemistry 85: 1165-1174.

Kreutzweiser, D.P. and Capell, D.P. (1996): Palatability of leaf material contaminated with Bacillus thuringiensis var. kurstaki, to Hydatophylax argus, a detritivorous aquatic insect. Bulletin of Environmental Contamination and Toxicology 56: 80-84. DOI:10.1007/s001289900012.

Kreutzweiser, D.P., Capell, S.S., Thomas, D.R. (1994): Aquatic insect responses to Bacillus thuringiensis var. kurstaki in a forest stream. Canadian Journal of Forest Research 24: 2041-2049. DOI:10.1139/cjfr-24-10-2041.

Kreutzweiser, D.P., Holmes, S.B., Capell, S.S., Eichenberg, D.C. (1992): Lethal and sublethal effects of Bacillus thuringiensis var kurstaki to aquatic insects in laboratory bioassays and outdoor stream channels. Bulletin of Environmental Contamination and Toxicology 49: 252-258. DOI: 10.1007/BF00191763.

Krogh, P.H. and Griffiths, B. (2007): ECOGEN: Soil ecological and economic evaluation of genetically modified crops. Pedobiologia 51: 171-173. DOI:10.1016/j.pedobi.2007.05.001.

Krogh, P.H., Griffiths, B., Demšar, D., Bohanec, M., Debeljak, M., Neumann Andersen, M., Sausse, C., Birch, A.N.E., Caul, S., Holmstrup, M., Heckmann, L.H., Cortet, J. (2007): Responses by earthworms to reduced tillage in herbicide tolerant maize and Bt maize cropping systems. Pedobiologia 51: 219-227. DOI:10.1016/j.pedobi.2007.04.003.

Kruger, G.R., Johnson, W.G., Weller, S.C., Owen, M.D.K., Shaw, D.R., Wilcut, J.W., Jordan, D.L., Wilson, R.G., Bernards, M.L., Young, B.G. (2009a): US grower views on problematic weeds and changes in weed pressure in glyphosate-resistant corn, cotton, and soybean cropping systems. Weed Technology 23, 162-166.

Kruger, M., Van Rensburg, J.B.J, Van den Berg, J. (2009b): Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa. Crop Protection 28, 684-689.

Kruger, M., Van Rensburg, J.B.J., Van den Berg, J. (2011a): Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa. Journal of Applied Entomology, DOI:10.1111/j.1439-0418.2011.01616.x.

Kruger, M., Van Rensburg, J.B.J., Van den Berg, J. (2011b): Resistance to Bt maize in Busseola fusca (Lepidoptera: Noctuidae) from Vaalharts. South Africa. Environmental Entomology 40, 477-483.

Kuiper, H.A., Davies, H.V. (2010): The SAFE FOODS Risk Analysis Framework suitable for GMOs? A case study. Food Control. DOI:10.1016/j.foodcont.2010.02.011.

Kwit, C., Moon, H.S., Warwick, S.I., Stewart, C.N. (2011): Transgene introgression in crop relatives: molecular evidence and mitigation strategies. Trends in Biotechnology 29 (6). DOI:10.1016/j.tibtech.2011.02.003.

Lamp, W. (2010): Risk assessment of transgenic corn debris on non-target arthropods in agricultural stream. ISI Information Systems for Biotechnology July, 5-8.

Landova, M., Hamouzove, K., Soukup, J., Holec, J., Squire, G.R. (2010): Population density and soil seed bank of weed beet as influenced by crop sequence and soil tillage. Plant, Soil and Environment 56 (11), 541-549.

Lang, A. (2004): Monitoring the impact of Bt maize on butterflies in the field: estimation of required sample sizes. Environmental Biosafety Research 3: 55-66.

Lang, A., Brunzel, S., Dolek, M., Otto, M., Theißen, B. (2011): Modelling in the light of uncertainty of key parameters: a call to exercise caution in field predictions of Bt-maize effects. Proceedings of the Royal Society. DOI:10.1098/rspb.2010.2085.

Lang, A., Ludy, C., Vojtech, E. (2004): Dispersion and deposition of Bt maize pollen in field margins. Z. Pflanzenkrankh. Pflanzensch. 111: 417-428.

Lang, A., Otto, M. (2010): A synthesis of laboratory and field studies on the effects of transgenic Bacillus thuringiensis (Bt) maize on non-target Lepidoptera. Entomologia Experimentalis et Applicata 135, 121-134. DOI:10.1111/j.1570-7458.2010.00981.x.

Lang, A. and Vojtech, E. (2006): The effects of pollen consumption of transgenic Bt maize on the common swallowtail, Papilo machaon L. (Lepidoptera; Papilionidae). Basic and Applied Ecology 7: 296-306. DOI:10.1016/j.baae.2005.10.003.

Lavigne, C., Klein, E.K., Couvet, D. (2002): Using seed purity data to estimate an average pollen mediated gene flow from crops to wild relatives. Theoretical and Applied Genetics 104: 139-145.

Lawo, N.C., Wäckers, F.L., Romeis, J. (2010): Characterizing indirect prey-quality mediated effects of a Bt crop on predatory larvae of the green lacewing, Chrysoperla carnea. Journal of Insect Physiology 56, 1702-1710. DOI:10.1016/j.jinsphys.2010.06.012.

Le Bail, M., Lecroart, B., Gauffretaeau, A., Angevin, F., Messean, A. (2010): Effect of the structural variables of landscapes on the risks of spatial dissemination between GM and non-GM maize. European Journal of Agronomy 33, 12-23. DOI:10.1016/j.eja.2010.02.002.

Lecomte, J., Bagger Jorgensen, R., Bartkowiak-Broda, I., Devaux, C., Dietz-Pfeilstetter, A., Gruber, S., Husken, A, Kuhlmann, M., Lutman, P., Rakousky, S., Sausse, C., Squire, G., Sweet, J., Aheto, D.W. (2007): Geneflow in oilseed rape: what the datasets of the UE Project SIGMEA tell us for coexistence? Proceedings GMCC07: Third International Conference on Coexistence between Genetically Modified and non-GM based agriculture supply chains. Luxembourg OOPEC. 49-52.

Lee, C.D., Penner, D., Hammerschmidt, R. (2003): Glyphosate and shade effects on glyphosate-resistant soybean defense response to Sclerotinia sclerotiorum. Weed Science Journal 51: 294-298. DOI:10.1614/0043-1745(2003)051[0294:GASEOG]2.0.CO;2.

Lee, T.N. and Snow, A.A. (1998): Pollinator preferences and the persistence of crop genes in wild radish populations (Raphanus raphanistrum, Brassicaceae). American Journal of Botany 85: 333-339.

Lefko, S.A., Nowatzki, T.M., Thompson, S.D., Binning, R.R., Pascual, M.A., Peters, M.L., Simbro, E.J., Stanley, B.H. (2008): Characterizing laboratory colonies of Western corn rootworm (Coleoptera: Chrysomelidae) selected for survival on maize containing event DAS-59122-7. Journal of Applied Entomology 132, 189-204.

Légère, A. (2005): Risks and consequences of gene flow from herbicide-resistant crops: canola (Brassica napus L) as a case study. Pest Management Science, 61: 292-300.

Lehman, R.M., Osborne, S.L., Prischmann-Voldseth, D.A., Rosentrater, K.A. (2010):. Insect-damaged corn stalks decompose at rates similar to Bt-protected, non-damaged corn stalks. Plant Soil 333, 481-490.

Lehman, R.M., Osborne, S.L., Rosentrater, K.A. (2008): No differences in decomposition rates observed between Bacillus thuringiensis and non- Bacillus thuringiensis corn residue incubated soil in the field. Agron. J. 100: 163-168.

Leroux, G.D., Chouinard, N., Nadeau, M., Buhler, S. (2006): Volet 3 – Aspects agroenvironnementaux. 1. Les cultures tolérantes aux herbicides. In: Michaud D et collaborateurs (Eds), Impact environnemental des cultures transgéniques cultivées au Québec, Ministère du Développement durable, de l'Environnement et des Parcs du Québec, Québec City, Canada, pp 107-128.

Lemaux, P.G. (2009): Genetically Engineered Plants and Foods: A Scientist's Analysis of the Issues (Part II). Annual Review of Plant Biology, 60: 511-559.

Leyval, C., Joner, E.J., Val, C. del, Haselwandter, K. (2002): Potential of arbuscular mycorrhizal fungi for bioremediation. In: Gianinazzi, S., Schuepp, H., Barea, J.M., Haselwandter, K. (Eds); Mycorrhizal technology in agriculture: 175-186. Birkhäuser. Basel.

Lheureux, K., Libeau-Dulos, M., Nilsagård H., Rodriguez Cerezo, E., Menrad, K., Menrad, M., Vorgrimler, D. (2003): Review of GMOs under research and development and in the pipeline in

Europe. In: European Commission, Joint Research Centre (DG JRC) Institute for Prospective Technological Studies (Eds); Technical Report Series EUR 20680 EN: 1-122.

Li, Y., Meissle, M., Romeis, J. (2008): Consumption of Bt maize pollen expressing Cry1Ab or Cry3Bb1 does not harm adult green lacewings, Chrysoperla carnea (Neuroptera: Chrysopidae). PLoS ONE 3: 1-8 (e2909).

Li, Y., Romeis, J. (2010): Bt maize expressing Cry3Bb1 does not harm the spider mite, Tetranychus urticae, or its ladybird beetle predator, Stethorus punctillum. Biological Control. DOI:10.1016/ j.biocontrol.2009.12.003.

Li, Y., Romeis, J., Wang, P., Peng, Y., Shelton, A.M. (2011): A Comprehensive Assessment of the Effects of Bt Cotton on Coleomegilla maculata Demonstrates No Detrimental Effects by Cry1Ac and Cry2Ab. PLoS ONE 6 (7). DOI:10.1371/journal.pone.0022185.

Lilley, A.K., Bailey, M.J., Cartwright, C., Turner, S.L., Hirsch, P.R. (2006): Life in earth: the impact of GM plants on soil ecology? Trends in Biotechnology 24: 9-14. DOI:10.1016/j.tibtech.2005.11.005.

Lima, M.A.P., Pires, C.S.S., Guedes, R.N.C., Nakasu, E.Y.T., Lara, M.S., Fontes, E.M.G., Sujii, E.R., Dias, S.C., Campos, L.A.O. (2010): Does Cry1Ac Bt-toxin impair development of worker larvae of Africanized honey bee? Journal of Applied Entomology. DOI:10.1111/j.1439-0418.2010.01573.x.

Lindfeld, A., Lang, C., Knop, E., Nentwig, W. (2011): Hard to digest or a piece of cake? Does GM wheat affect survival and reproduction of Enchytraeus albidus (Annelida: Enchytraeidae)? Applied Soil Ecology 47, 51-58. DOI:10.1016/j.apsoil.2010.10.012.

Liu, W. (2010): Do genetically modified plants impact arbuscular mycorrhizal fungi? Ecotoxicology 19, 229–238. DOI:10.1007/s10646-009-0423-1.

Liu, X., Chen, M., Onstad, D., Roush, R., Shelton, A.M. (2011): Effect of Bt broccoli and resistant genotype of Plutella xylostella (Lepidoptera: Plutellidae) on development and host acceptance of parasitoid Diadegma insulare (Hymenoptera: Ichneumonidae): Transgenic Research 20, 887-897. DOI:10.1007/s11248-010-9471-9.

Liu, X.D., Zhai, B.P., Zhang, X.X., Zong, J.M. (2005): Impact of transgenic cotton plants on a non-target pest; Aphis gossypii Glover. Ecological Entomology 30: 307-315.

Liu, Z., Gao, Y., Luo, J., Lai, F. Li, Y. Fu, Q., Peng, Y. (2011): Evaluating the Non-Rice Host Plant Species of Sesamia inferens (Lepidoptera: Noctuidae) as Natural Refuges: Resistance Management of Bt Rice. Environmental Entomology 40 (3), 749-754. DOI:10.1603/EN10264.

Llorente, B., Alonso, G.D., Bravo-Almonacid, F., Rodriguez, V., Lopez, M.G., Carrari, F., Torres, H.N., Flawia, M.M. (2010): Safety assessment of nonbrowning potatoes: opening the discussion about the relevance of substantial equivalence on next generation biotech crops. Plant Biotechnology Journal, 1-15. DOI:10.1111/j.1467-7652.2010.00534.x.

Lobell, D.B., Field, C.B. (2008): Estimation of the carbon dioxide (CO2) fertilization effect using growth rate anomalies of CO2 and crop yields since 1961. Global Change Biology 14: 39-45. DOI: 10.1111/j.1365-2486.2007.01476.x.

Londo, J.P., Bautista, N.S., Sagers, C.L., Lee, E.H., Watrud, L.S. (2010): Glyphosate drift promotes changes in fitness and transgene gene flow in canola (Brassica napus) and hybrids. Annals of Botany 106: 957-965.

Lopez, M.D., Sumerford, D.V., Lewis, L.C. (2010): Nosema pyrausta and Cry1Ab-incorporated diet led to decreased survival and developmental delays in European corn borer. Entomologia Experimentalis et Applicata 134, 146-153. DOI:10.1111/j.1570-7458.2009.00947.x.

Lorenz, M.G., Wackernagel, W. (1994): Bacterial gene transfer by natural genetic transformation in the environment. Microbiological Reviews (now published as Microbiology and Molecular Biology Reviews) 58: 563-602.

Losey, J.E., Rayor, L.S., Carter, M.E. (1999): Transgenic pollen harms monarch larvae. Nature 399: 214.

Loureiro, I., Escorial, C., Garcia-Baudin, J.M., Chueca, M.C. (2009): Spontaneous wheat – Aegilops biuncialis, Ae. geniculata and Ae. triuncialis amphiploid production, a potential way of gene transference. Spanish Journal of Agricultural Research , 7 , (3) , p.614-620.

Lövei, G.L., Arpaia, S. (2005): The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomologia Experimentalis et Applicata 114: 1-14.

Lozzia, G.C. (1999): Biodiversity and structure of ground beetle assemblages (Coleoptera Carabidae) in Bt corn and ist effects on non target insects. Bollettino di Zoologia Agraria e di Bachicoltura Ser II 31: 37-58.

Lu, B.R. (2005): Multidirectional gene flow among wild, weedy, and cultivated soybeans. In: Gressel, J. (Ed); Crop ferality and volunteerism: 137-147. CRC Press.

Lu, Y., Wu, K., Jiang, Y., Xia, B., Li, P., Feng, H., Wyckhuys, K.A., Guo, Y. (2010): Mired bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. Scienceexpress.

Ludy, C., and Lang, A. (2006): A 3-year field-scale monitoring of foliage-dwelling spiders (Araneae) in transgenic Bt maize fields and adjacent field margins. Biological Control 38: 314-324. DOI:10.1016/j.biocontrol.2006.05.010.

Luijten, S.H. and de Jong, T,J. (2011) Hybridisation and introgression between Brassica napus and B. rapa in the Netherlands. CGM report 2011-06, available at http://cogem.net

Lumbierres, B., Albajes, R., Pons, X. (2004): Transgenic Bt maize and Rhopalosiphum padi (Hom., Aphididae) performance. Ecological Entomology 29: 309-317.

Lumbierres, B., Stary, P., Pons, X. (2010): Effect of Bt maize on the plant-aphid-parasitoid tritrophic Relationships. BioControl. DOI:10.1007/s10526-010-9317-7.

Luna V.S., Figueroa M.J., Baltazar M.B., Gomez R.L., Townsend, R., Schoper, J.B. (2001): Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Science 41: 1551-1557.

Lundgren, J.G. and Wiedemann, S. (2002): Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a nontarget species Coleomegilla maculata DeGeer (Coleoptera: Coccinellidae). Environmental Entomology 31: 1213-1218.

Lundgren, J.G., Huber, A., Wiedenmann, R.N. (2005): Quantification of consumption of corn pollen by the predator Coleomegilia maculata (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield. Agricultural and Forest Entomology 7: 53-60.

Lundgren, J.G. and Wiedenmann, R.N. (2005): Tritrophic interactions among Bt (Cry3Bb1) corn, aphid prey, and the predator Coleomegilla maculata (Coleoptera: Coccinellidae). Environmental Entomology, 34: 1621-1625.

Lutman, P., Berry, K., Payne, R., Simpson, E., Sweet, J., Champion, G., May, M., Wightman, P., Walker, K., Lainsbury, M. (2005a): An update on the persistence of seeds from crops of conventional and genetically modified herbicide tolerant oilseed rape (Brassica napus). Proceedings of 2nd International conference on Coexistence between GM and non-GM based agricultural supply chains, Montpellier 2005, 93-96.

Lutman, P., Berry, K., Payne, R., Simpson, E., Sweet, J., Champion, G., May, M., Wightman, P., Walker, K., Lainsbury, M. (2005b): Persistence of seeds from crops of conventional and genetically modified herbicide tolerant oilseed rape (Brassica napus). Proc. Royal Soc. London B. 272.

Lutman, P.J.W., Sweet, J., Berry, K., Law, J., Payne, R., Simpson, E., Walker, K., Wightman, P. (2008): Weed control in conventional and herbicide tolerant winter oilseed rape (Brassica napus) grown in rotations with winter cereals in the UK. Weed Research, 48: 408-419.

Ma, B.L., Subedi, K.D., Reid, L.M. (2004): Extent of cross-fertilization in maize by pollen from neighboring transgenic hybrids. Crop Science 44: 1273-1282.

Madsen, K.H. and Jensen, J.E. (1995): Weed control in glyphosate-tolerant sugar-beet (Beta vulgaris L.). Weed Research, 35: 105-111.

Madlinger, M., Gasser, C.A., Schwarzenbach, R.P., Sander, M. (2011): Adsorption of Transgenic Insecticidal Cry1Ab Protein to Silica Particles. Effects on Transport and Bioactivity. Environmental Science and Technology 45, 4377-4384. DOI:10.1021/es200022q.

Magnussen, L.S. and Hauser, T.P. (2007): Hybrids between cultivated and wild carrots in natural populations in Denmark. Heredity 99, 185–192.

Mallory-Smith, C.A. and Zapiola, M.L. (2008): Gene flow from glyphosate-resistant crops. Pest Management Sci. 64:428-440.

Malone, L. (2004): Potential effects of GM crops on honey bee health. Bee World (now Journal of Apicultural Research) 85: 29-36.

Malone, L.A. and Burgess, E.P.J. (2009): Impact of genetically modified crops on pollinators. In: Ferry, N., Gate-house, A.M.R. (Eds.), Environmental Impact of Genetically Modified Crops, CAB International, pp. 199-222.

Malone, L.A., Todd, J.H., Burgess, E.P.J., Christeller, J.T. (2004): Development of hypopharyngeal glands in adult honey bees fed with a Bt toxin, a biotin-binding protein and a protease inhibitor. Apidologie 35: 655-664. DOI:10.1051/apido:2004063.

Malone, L.A., Burgess, E.P.J., Gatehouse, H.S., Voisey, C.R., Tregidga, E.L., Philip, B.A. (2001): Effects of ingestion of a Bacillus thuringiensis toxin and a trypsin inhibitor on honey bee flight activity and longevity. Apidologie 32: 57-68.

Malone, L.A., Burgess, E.P.J., Stefanovic, D. (1999): Effects of a Bacillus thuringiensis toxin, two Bacillus thuringiensis biopesticide formulations, and a soybean trypsin inhibitor on honey bee (Apis mellifer L.) survival and food consumption. Apidologie 30: 465-473.

Mamy, L., Gabrielle, B., Barriuso, E. (2010): Comparative environmental impacts of glyphosate and conventional herbicides when used with glyphosate-tolerant and non-tolerant crops. Environmental Pollution 158, 3172-3178.

Manachini, B., Landi, S., Fiore, M.C., Festa, M., Arpaia, S. (2004): First investigations on the effects of Bt-transgenic Brassica napus L. on the trophic structure of the nematofauna. IOBC wprs Bulletin 27: 103-108.

Marchetti, E., Accinelli, C., Talamè, V., Epifani, R. (2007): Persistence of Cry toxins and cry genes from genetically modified plants in two agricultural soils. Agron. Sustain. Develop. 27: 231-236.

Marroquin, L.D., Elyassnia, D., Griffiths, J.S., Feitelson, J.S., Aroian, R.V. (2000): Bacillus thuringiensis (Bt) toxin susceptibility and isolation of resistance mutants in the nematode Caenorhabditis elegans. Genetics 155: 1693-1699.

Marshall, E.J.P., Brown, V.K., Boatman, N.D., Lutman, P.J.W., Squire, G.R., Ward, L.K. (2003): The role of weeds in supporting biological diversity within crop fields. Weed Research 43: 77-89.

Marshall, J.M. (2010): The Cartagena Protocol and genetically modified mosquitoes. Nature Biotechnology 28 (9), 896-897.

Marvier, M. (2011): using meta-analysis to inform risk assessment and risk management. Journal für Verbraucherschutz und Lebensmittelsicherheit. DOI:10.1007/s00003-011-0675-6.

Marvier, M., McCreedy, C., Regetz, J., Kareiva, P. (2007): A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science 316: 1475-1477. DOI:10.1126/science.1139208.

Masclaux, H., Bec, A., Kagami, M., Perga, M.E., Sime-Ngando, T., Desvilettes, C., Bourdier, G. (2011): Food quality of anemophilous plant pollen for zooplankton. Limnology and Oceanography 56, 939-946.

Masoero, F.M., Moschini, F., Rossi, A., Prandini, A., Pietri, A. (1999): Nutritive value, mycotoxin contamination and in vitro rumen fermentation of normal and genetically modified corn (cry1A(b)) grown in northern Italy. Maydica 44: 205-209.

Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J. (1997): Agricultural intensification and ecosystem properties. Science 277: 504-509.

Matten, S.R., Head, G.P., Quemada, H.D. (2008): How governmental regulation can help or hinder the integration of Bt crops into IPM programs. In: Romeis J., Shelton A.M., Kennedy G.G. (Eds), Integration of Insect-Resistant Genetically Modified Crops within IPM Programs, Springer Science+ Business Media BV, pp 27-39.

May, M.J., Champion, G.T., Dewar, A.M., Qi, A., Pidgeon, J.D. (2005): Management of genetically modified herbicide tolerant sugar beet for spring and autumn environmental benefit. Proceedings of the Royal Society B-Biological Sciences, 272: 111-119.

McGaughey, W.H. (1985): Insect resistance to the biological insecticide Bacillus thuringiensis. Science 229, 193-195.

McGinnis, E., Meyer, M.H., Smith, A.G. (2010): Sweet and Sour: A Scientific and Legal Look at Herbicide-Tolerant Sugar Beet. The Plant Cell 22, 1653-1657; DOI:10.1105/tpc.110.077198.

McPartlan, H.C. and Dale, P.J. (1994): An assessment of gene transfer by pollen from field-grown transgenic potatoes to non-transgenic potatoes and related species. Transgenic Research 3: 216-225.

Means, N.E., Kremer, R.J., Ramsier, C. (2007): Effects of glyphosate and foliar amendments on activity of microorganisms in the soybean rhizosphere. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes, 42: 125-132.

Meihls, L.N., Higdon, M.L., Ellersieck, M., Hibbard, B.E. (2011): Selection for Resistance to mCry3A-Expressing Transgenic Corn in Western Corn Rootworm. Journal of Economic Entomology 104 (3), 1045-1054. DOI:10.1603/EC10320.

Meihls, L.N., Higdon, M.L., Siegfried, B.D., Miller, N.J., Sappington, T.W., Ellersieck, M.R., Spencer, T.A., Hibbard, B.E. (2008): Increased survival of Western corn rootworm on transgenic corn within three generations of on-plants greenhouse selection. Proceedings of the National Academy of Sciences of the United States of America 105, 19177-19182.

Meissle, M. and Lang, A. (2005): Comparing methods to evaluate the effects of Bt maize and insecticide on spider assemblages. Agriculture, Ecosystems and Environment 107: 359-370. DOI:10.1016/j.agee.2004.12.007.

Meissle, M., Mouron, P., Musa, T., Bigler, F., Pons, X., Vasileiadis, V.P., Otto, S., Antichi, D., Kiss, J., Pálinkás, Z., Dorner, Z., van der Weide, R., Groten, J., Czembor, E., Adamczyk, J., Thibord, J.-B., Melander, B., Nielsen, G.C., Poulsen, R.T., Zimmermann, O., Verschwele, A., Oldenburg, E. (2010): Pests, pesticide use and alternative options in European maize production: current status and future prospects. Journal of Applied Entomology, 1-19. DOI:10.1111/j.1439-0418.2009.01491.x.

Meissle, M., Romeis, J. (2009): Insecticidal activity of Cry3Bb1 expressed in Bt maize on larvae of the Colorado potato beetle, Leptinotarsa decemlineata. Entomologia Experimentalis et Applicata 131: 308-319.

Meissle, M., Romeis, J., Bigler, F. (2011): Bt maize and integrated pest management – a European perspective. Pest Management Science. DOI:10.1002/ps.2221.

Meissle, M., Vojtech, E., Poppy, G.M. (2005): Effects of Bt maize-fed prey on the generalist predator Poecilus cupreus L. (Coleoptera: Carabidae). Transgenic Research 14: 123-132. DOI:10.1007/s11248-004-6458-4.

Messéan, A., Squire, G., Perry, J., Angevin, F., Gomez, M., Townend, P. Sausse, C., Breckling, B., Langrell, S., Dzeroski, S., Sweet J. (2009): Sustainable introduction of GM crops into European agriculture: a summary report of the FP6 SIGMEA research project . Oléagineux, Corps Gras, Lipides. Volume 16, Numéro 1, 37-51, Agronomie – Environnement. DOI : 10.1684/ocl.2009.0241.

Meyer, H. (2011): Systemic risks of genetically modified crops: the need for new approaches to risk assessment. Environmental Sciences Europe 23 (7). DOI:10.1186/2190-4715-23-7.

Miethling-Graff, R., Dockhorn, S., Tebbe, C.C. (2010): Release of the recombinant Cry3Bb1 protein of Bt maize MON88017 into field soil and detection of effects on the diversity of rhizosphere bacteria. European Journal of Soil Biology 46: 41-48.

Mikkelsen, T.R., Andersen, B., Jørgensen, R.B. (1996): The risk of crop transgene spread. Nature 380: 31.

Miller, J.K., Bradford, K.J. (2010): The regulatory bottleneck for biotech specialty crops. Nature Biotechnology 28 (10).

Mizuguti, A., Phigashi, K., Yoshimura, Y., Kaga, A., Kurode, Y., Matsudo, K. (2010): Hybridization between GM soybean (Glycine max (L.) Merr.) and wild soybean (Glycine soja Sieb. et Zucc.) under field conditions in Japan. Environ. Biosafety Res., DOI:10.1051/ebr/2010004.

Moar, W., Roush, R., Shelton, A., Ferré, J., MacIntosh, S., Leonard, B.R., Abel, C. (2008): Field-evolved resistance to Bt toxins. Nat. Biotechnol. 26: 1072-1074.

Mohr, K.I., Tebbe, Ch. (2007): Field study results on the probability and risk of a horizontal gene transfer from transgenic herbicide-resistant oilseed rape pollen to gut bacteria of bees. Applied Microbiology and Biotechnology 75: 573-582. DOI:10.1007/s00253-007-0846-7.

Monier, J.M., Bernillon, D., Kay, E., Faugier, A., Rybalka, O., Dessaux, Y., Simonet, P., Vogel, T. (2007): Detection of potential transgenic plant DNA recipients among soil bacteria. Environmental Biosafety Research 6: 71-83. DOI:10.1051/ebr:2007036.

Monsanto (2007a): Roundup PRO2 – Herbicide – Formulation concentre soluble – Technical Guide France.

Monsanto (2007b): Cuaderno technico No6 “Seguridad del Herbicida roundup Ready® y su empleo sobre variedades modificadas geneticamente para tolerancia a glifosato” Technical Guide Spain.

Montagu Van, M. (2011): It Is a Long Way to GM Agriculture. Annual Review of Plant Biology, 62, 1-23. DOI:10.1146/annurev-arplant-042110-103906.

Moorman, T.B. (1989): A review of pesticide effects on microorganisms and microbial processes related to soil fertility. Journal of Production Agriculture 2: 14-23.

Moorman, T.B., Becerill, J.M., Lydon, J., Duke, S.O. (1992): Production of hydrobenzoic acids by Bradyrhizobium japonicum strains treatment with glyphosate. Journal of Agricultural Food chemistry 40: 289-293.

Morris, S.H. (2007): EU biotech crop regulations and environmental risk: a case of the emperor’s new clothes? Trends in Biotechnology 25, 2-6.

Motavalli, P.P., Kremer, R.J., Fang, M., Means, N.E. (2004): Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. Journal of Environmental Quality 33: 816-824.

Mulder, C., Wouterse, M., Raubuch, M., Roelofs, W., Rutgers, M. (2006): Can transgenic maize affect soil microbial communities? Public Library of Science (PLoS): Computational Biology: e128. DOI:10.1371/journal.pcbi.0020128.

Mullin, C.A., Saunders, M.C., Leslie, T.W., Biddinger, D.J., Fleischer, S.J. (2005): Toxic and behavioral effects to Carabidae of seed treatments used on Cry3Bb1- and Cry1Ab/c-protected corn. Environmental Entomology 34: 1626-1636.

Murphy, A.F., Ginzel, M.D., Krupke, C.H. (2010): Evaluating western corn rootworm (Coleoptera: Chrysomelidae) emergence and root damage in a seed mix refuge. Journal of Economic Entomology, 103, 147-157.

Myers, M.W., Curran, W.S., VanGessel, M.J., Majek, B.A., Scott, B.A., Mortensen, D.A., Calvin, D.D., Karsten, H.D., Roth, G.W. (2005): The effects of weed density and application timing on weed control and corn grain yield. Weed Technology, 19: 102-107.

Myhr, A.I. (2010): A Precautionary Approach to Genetically Modified Organisms: Challenges and Implications for Policy and Science. Journal of Agricultural and Environmental Ethics. DOI:10.1007/s10806-010-9234-x.

NABU (2010): Welche Rechte hat der Natur- und Artenschutz beim Anbau gentechnisch veränderter Nutzpflanzen? Gentechnikrecht & Artenschutz.

Naranjo, S.E. (2005a): Long-term assessment of the effects of transgenic Bt cotton on the function of the natural enemy community. Environmental Entomology 34: 1211-1223.

Naranjo, S.E. (2005b): Long-term assessment of the effects of transgenic Bt cotton on the abundance of nontarget natural enemies. Environmental Entomology 34: 1193-1210.

Naranjo, S.E. (2009): Impacts of Bt crops on non-target invertebrates and insecticide use patterns. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 4: 1-23.

Naranjo, S.E., Ruberson, J.R., Sharma, H.C., Wilson, L., Wu, K. (2008): The present and future role of insectresistant genetically modified cotton in IPM. In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), Integration of insect-restistant genetically modified crops within IPM programs, Springer Science +Business Media BV, pp. 159-194.

National Research Council (2010): The Impact of Genetically Engineered Crops on Farm Sustainability in the United States. National Academy of Sciences: 1-254.

Nelson, K.A. and Renner, K.A. (2001): Soybean growth and development as affected by glyphosate and postemergence herbicide tank mixtures. Agronomy Journal 93, 428-434.

Nelson, G.C. and Bullock, D.S. (2003): Simulating a relative environmental effect of glyphosate-resistant soybeans. Ecological Economics 45, 189-202.

Neve, P. (2008): Simulation modeling to understand the evolution and management of glyphosate resistant weeds. Pest Management Science 64, 392-401.

Neve, P., Sadler, J., Powles, S.B. (2004): Multiple herbicide resistance in a glyphosate-resistant rigid ryegrass (Lolium rigidum) population. Weed Science 52, 920-928.

Nguyen, H.T. and Jehle, J.A. (2007): Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. Journal of Plant Diseases and Protection 114: 82-87.

Nielsen, K.M., Bones, A.M., Smalla, K., Elsas, J.D. van (1998): Horizontal gene transfer from transgenic plants to terrestrial bacteria – a rare event? FEMS Microbiol. Rev. (FEMS Microbiology Reviews) 22: 79-103.

Nielsen, K.M., Johnson, P.J., Bensasson, D., Daffonchio, D. (2007): Release and persistence of extracellular DNA in the environment. Environmental Biosafety Research 6: 37-53. DOI: 10.1051/ebr:2007031.

Nielsen, K.M. and Townsend, J.P. (2004): Monitoring and modeling horizontal gene transfer. Nature Biotechnology 22: 1110-1114. DOI:10.1038/nbt1006.

Nielsen, K.M., Weerelt, D.M. van, Berg, T.N., Bones, A.M., Hageler, A.N., Elsas, J.D. van (1997): Natural transformation and availability of transforming chromosomal DNA to Acinetobacter calcoaceticus in soil microcosms. Applied and Environmental Microbiology 63: 1945-1952.

Njiti, V.N., Myers, O.Jr., Schroeder, D., Lightfood, D.A. (2003): Roundup Ready soybean: glyphosate effects on Fusarium solani root colonization and sudden death syndrome. Agronomy Journal 95: 1140-1145.

Norris, C. and Sweet, J. (2002): Monitoring large scale releases of genetically modified crops (EPG1/5/84) incorporating report on project EPG 1/5/30: monitoring releases of genetically modified crop plants, DEFRA report, EPG 1/5/84. http://www.defra.gov.uk/environment/gm/research/pdf/epg_1-5-84_screen.pdf

Norsworthy, J.K. (2008): Effect of tillage intensity and herbicide programs on changes in weed species density and composition in the southeastern coastal plains of the United States. Crop Protection 27: 151-160.

Nowatzki, T.M., Lefko, S.A., Binning, R.R., Thompson, S.D., Spencer, T.A., Siegfried, B.D. (2008): Validation of a novel resistance monitoring technique for corn rootworm (Coleoptera: Chrysomelidae) and event DAS-59122-7 maize. Journal of Applied Entomology 132, 177-188.

Oberhauser, K.S., Prysby, M.D., Mattila, H.R., Stanley-Horn, D.E., Sears, M.K., Dively, G., Olson, E., Pleasants, J.M., Lam, W.K.F., Hellmich, R.L. (2001): Temporal and spatial overlap between monarch larvae and corn pollen. Proceedings of the National Academy of Sciences of the USA 98: 11913-11918. DOI:10.1073/pnas.211234298.

Obrist, L.B., Dutton, A., Albajes, R., Bigler, F. (2006a): Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. Ecological Entomology 31: 143-154.

Obrist, L.B., Dutton, A., Romeis, J., Bigler, F. (2006b): Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator Chrysoperla carnea. BioControl 51: 31-48. DOI:10.1007/s10526-005-2936-8.

Obrist, L.B., Dutton, A., Romeis, J., Bigler, F. (2006c): Fate of Cry1Ab toxin expressed by Bt maize upon ingestion by herbivorous arthropods and consequences for Chrysoperla carnea. BioControl 51, 31-48.

Obrist, L.B., Klein, H., Dutton, A., Bigler, F. (2005): Effects of Bt maize on Frankliniella tenuicornis and exposures of thrips predators to prey-mediated Bt toxin. Entomologia Experimentalis et Applicata 115: 409-416. DOI:10.1111/j.1570-7458.2005.00298.x.

O'Callaghan, M., Glare, T.R., Burgess, E.P.J., Malone, L.A. (2005): Effects of plants genetically modified for insect resistance on nontarget organisms. Annual Review of Entomology 50: 271-292. DOI:10.1146/annurev.ento.50.071803.130352.

OECD (1997): Consensus document on the biology of Solanum tuberosum spp. tuberosum (potatoe) Series on Harmonisation of Regulatory Oversight in Biotechnology 8.

OECD (2003): Consensus document on the biology of Zea mays subsp. mays (Maize) Series on Harmonisation of Regulatory Oversight in Biotechnology 27. URL: http://www.oecd.org/findDocument/0,3354,en_2649_34385_1_1_1_1_37437,00.html

OECD (2006): Safety assessment of transgenic organisms: Consensus documents on the biology of trees. OECD Consensus Documents Volume 2 (1996-2006).

OECD (2007): Consensus document on safety information on transgenic plants expressing Bacillus thuringiensis – derived insect control proteins. Series on Harmonisation of Regulatory Oversight in Biotechnology.

Oehen, B., Costa-Font, M., Morgner, M., Gil, J.M., Stolze, M. (2007): Co-existence in maize supply chains in Spain and Switzerland. 3rd QLIF Congress, Hohenheim, Germany, March 20-23, 2007. http://orgprints.org/10379/01/oehen-etal-2007-Co_existence_maize.pdf

Oehen, B., Stolze, M. (2009): Die Kosten der Koexistenz von gentechnisch veränderten und biologischen Kulturen: Fallbeispiele aus Frankreich und der Grenzregion. Beitrag präsentiert bei der Konferenz: 10. Wissenschaftstagung Ökologischer Landbau, Zürich, 11.-13. Februar 2009. http://orgprints.org/14501/01/Oehen_14501.pdf

Onstand; D.W., Mitchell, P.D., Hurley, T.M., Lundgren, J.G., Porter, R.P., Krupke; C.H., Spencer; J.L., Difonzo, C.D., Baute, T.S., Hellmich, R.L., Buschmann, L.L., Hutchison, W.D., Tooker, J.F. (2011): Seeds of Change: Corn Seed Mixtures for Resistance Management and Integrated Pest Management. Journal of Economic Entomology, 104 (2), 343-352. DOI:10.1603/EC10388.

Oswald, K.J., Wade French, B., Nielson, C., Bagley, M. (2011): Selection for Cry3Bb1 Resistance in a Genetically Diverse Population of Nondiapausing Western Corn Rootworm (Coleoptera: Chrysomelidae): Journal of Economic Entomology 104 (3), 1038-1044. DOI:101603/EC10312.

Otsu, Y., Mori, H., Komuta, K., Shimizu, H., Nogawa, S., Matsuda, Y., Nonomura, T., Sakuratani, Y., Tosa, Y., Mayama, S., Toyoda, H. (2003): Suppression of leaf feeding and oviposition of phytophagous ladybird beetles (Coleoptera: Coccinelidae) by chitinase gene-transformed Phylloplane bacteria and their specific bacteriophages entrapped in alginate gel bands. Journal of Economic Entomology 96: 555-563.

Owen, M.D.K. (2005): Maize and Soybeans - controllable volunteerism without ferality? In: Gressel, J. (Ed); Crop ferality and volunteerism: 149-165. CRC Press.

Owen, M.D.K. (2010): Herbicide Resistance. Genetic Modification of Plants, Biotechnology in Agriculture and Forestry 64, 159-176. DOI:10.1007/978-3-642-02391-0_9.

Owen, M.D.K. (2011): Weed resistance development and management in herbicide-tolerant crops: experiences from the USA. Journal für Verbraucherschutz und Lebensmittelsicherheit. DOI: 10.1007/s00003-011-0679-2.

Owen, M.D.K., Pedersen, P., De Bruin, J.L., Stuart, J., Lux, J., Franzenburg, D., Grossnickle, D. (2010): Comparisons of Genetically Modified and Non-Genetically Modified Soybean Cultivars and Weed Management Systems. Crop Science 50, 2597–2604. DOI:10.2135/cropsci2010.01.0035.

Owen, M.D.K., Young, B.G., Shaw, D.R., Wilson, R.G., Jordan, D.L., Dixon, P.M., Weller, S.C. (2011): Benchmark study on glyphosate-resistant crop systems in the United States. Part 2: Perspectives. Pest Management Science. DOI:10.1002/ps.2159.

Owen, M.D.K., Zelaya, I.A. (2005): Herbicide-resistant crops and weed resistance to herbicides. Pest Management Science 61: 301-311. DOI:10.1002/ps.1015.

Pagel-Wieder, S., Niemeyer, J., Fischer, W.R., Gessler, F. (2007): Effects of physical and chemical properties of soils on adsorption of the insecticidal protein (Cry1Ab) from Bacillus thuringiensis at Cry1Ab protein concentrations relevant for experimental field sites. Soil Biology & Biochemistry 39: 3034-3042. DOI:10.1016/j.soilbio.2007.06.015.

Pálinkás, Z., Zalai, M., Szénási, A., Dorner, Z., Szekeres, D. (2012): Botanical and arthropod diversity in GM HT maize treated with glyphosate or conventional herbicides. IOBC wprs Bulletin.

Papazova, N., Zhang, D., Gruden, K., Vojvoda, J., Yang, L., Gasparic, M.B., Blejec, A., Fouilloux, S., de Loose, M., Taverniers, I. (2010): Evaluation of the reliability of maize reference assays for GMO quantification. Analytical and Bioanalytical Chemistry 396, 2189-2201. DOI:10.1007/s00216-009-3386-4.

Parker, R.G., York, A.C., Jordan, D.L. (2006): Weed control in glyphosate-resistant corn as affected by preemergence herbicide and timing of postemergence herbicide application. Weed Technology, 20: 564-570.

Parrot, W. (2008): Study of Bt impact on caddisflies overstates its conclusions: Response to Rosi-Marshall et al., Proc. Natl. Acad. Sci. USA 105: E10.

Parrott, W., Chassy, B., Ligon, J., Meyer, L., Petrick, J., Zhou, J., Herman, R., Delaney, B., Levine, M. (2010): Application of food and feed safety assessment principles to evaluate transgenic approaches to gene modulation in crops. Food and Chemical Toxicology. DOI:10.1016/j.fct.2010.04.017.

Pascher, K., Macalka, S., Rau, D., Gollmann, G., Reiner, H., Glossl, J., Grabherr, G. (2010): Molecular differentiation of commercial varieties and feral populations of oilseed rape (Brassica napus L.): BMC Evolutionary Biology 10 (63), 1-13.

Pascher, K., Moser, D., Dullinger, S., Sachslehner, L., Gros, P., Sauberer, N., Traxler, A., Grabherr, G., Frank, T. (2011): Setup, efforts and practical experiences of a monitoring program for genetically modified plants – An Austrian case study for oilseed rape and maize. Environmental Sciences Europe 23 (12). DOI:10.1186/2190-4715-23-12.

Pasonen, H.L., Degefu, Y., Brumós, J., Lohtander, K., Pappinen, A., Timonen, S., Seppänen, S. K. (2005): Transgenic Betula pendula expressing sugar beet chitinase IV forms normal ectomycorrhizae with Paxillus involutus in vitro. Scandinavian Journal of Forest Research 20: 385-392.

Paul, L., Angevin, F., Collonnier, C., Messéan, A. (2011): Impact of gene stacking on gene flow: the case of maize. Transgenic Research. DOI:10.1007/s11248-011-9527-5.

Pengue, W.A. (2005): Transgenic crops in Argentina: The ecological and social debt. Bulletin of Science, Technology & Society 25: 314-322. DOI:10.1177/0270467605277290.

Pereira, E.J.G., Storer, N.P., Siegfried, B.D. (2010): Fitness costs of Cry1F resistance in laboratory-selected European corn borer (Lepidoptera: Crambidae): Journal of Applied Entomology, 1-8. DOI:10.1111/j.1439-0418.2009.01488.x.

Perry, J.N., Devos, Y., Arpaia, S., Bartsch, D., Ehlert, C., Gathmann, A., Hails, R.S., Hendriksen, N.B., Kiss, J., Messéan, A., Mestdagh, S., Neemann, G., Nuti, M., Sweet, J.B., Tebbe, C.C. (2011b): Estimating the effects of Cry1F Bt-maize pollen on non-target Lepidoptera using a mathematical model of exposure. Journal of Applied Ecology 49 (1): 29-37. DOI:10.1111/j.1365-2664.2011.02083.x.

Perry, J.N., Devos, Y., Arpaia, S., Bartsch, D., Gathmann, A., Hails, R.S., Kiss, J., Lheureux, K., Manachini, B., Mestdagh, S., Neemann, G., Ortego, F., Schiemann, J., Sweet, J.B. (2010): A mathematical model of exposure of non-target Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe. Proceedings of the Royal Society. DOI:10.1098/rspb.2009.2091.

Perry, J.N., Devos, Y., Arpaia, S., Bartsch, D., Gathmann, A., Hails, R.S., Kiss, J., Lheureux, K., Manachini, B., Mestdagh, S., Neemann, G., Ortego, F., Schiemann, J., Sweet, J.B. (2011a): The usefulness of a mathematical model of exposure for environmental risk assessment. Proceedings of the Royal Society. DOI:10.1098/rspb.2010.2667.

Perry, J.N., Firbank, L.G., Champion, G.T., Clark, S.J., Heard, M.S., May, M.J., Hawes, C., Squire, G.R., Rothery, P., Woiwod, I.P., Pidgeon, J.D. (2004): Ban on triazine herbicides likely to reduce but not negate relative benefits of GMHT maize cropping. Nature 428: 313-316. DOI:10.1038/-nature02374.

Perry, J.N., Rothery, P., Clark, S.J., Heard, M.S., Hawes, C. (2003): Design, analysis and power of the Farm-Scale Evaluations of genetically modified herbicide tolerant crops. Journal of Applied Ecology, 40: 17-31.

Pertl, M., Hauser, T.P., Damgaard, C., Jørgensen, R.B. (2002): Male fitness of oilseed rape (Brassica napus), weedy B. rapa and their F1-hybrids when pollinating B. rapa seeds. Heredity 89: 212-218.

Pertoldi, C., Bijlsma, R., Loeschcke, V. (2007): Conservation genetics in a globally changing environment: Present problems, paradoxes and future challenges. Biodiversity and Conservation 16: 4147-4163. DOI:10.1007/s10531-007-9212-4.

Peter, M., Lindfeld, A., Nentwig, W. (2010): Does GM wheat affect saprophagous Diptera species (Drosophilidae, Phoridae)? Pedobiologia. DOI:10.1016/j.pedobi.2009.12.006.

Peterson, R.K.D and Hulting, A.G. (2004): A comparative ecological risk assessment for herbicides used on spring wheat: the effect of glyphosate when used within a glyphosate-tolerant wheat system. Weed Science 52, 834-844.

Petit, S., Boursault, A., Le Guilloux, M., Munier-Jolain, N., Reboud, X. (2010): Weeds in agricultural landscapes. A review. Agronomy for Sustainable Development. DOI:10.1051/agro/2010020.

Pichersky, E. and Gershenzon, J. (2002): The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Current Opinion in Plant Biology 5: 237-243. DOI:10.1016/S1369-5266(02)00251-0.

Pidgeon, J.D., May, M.J., Perry, J.N., Poppy, G.M. (2007): Mitigation of indirect environmental effects of GM crops. Proceedings of the Royal Society B-Biological Sciences, 274: 1475-1479.

Pierre, J., Pham-Delègue, M.H. (2000): Comment étudier l´impact de colzas transgéniques sur les abeilles? Oleagineux Corps Gras Lipides 7: 341-344.

Piesse, J., Thirtle, C. (2010): Agricultural R&D, technology and productivity. Philosophical Transaction of the Royal Society 365, 3035-3047. DOI:10.1098/rstb.2010.0140.

Pigott, C.R. and Ellar, D.J. (2007): Role of receptors in Bacillus thuringiensis crystal toxin activity. Microbiol. Mol. Biol. Rev. 71: 255-281.

Pilate, G., Guiney, E., Holt, L., Petit-Conil, M., Lapierre, C., Leplé, J-C., Pollet, B., Mila, I., Webster, E. A., Marstorp, H.G., Hopkins, D.W., Jouanin, L., Boerjan, W., Schuch, W., Cornu, D., Halpin, C. (2002): Field and pulping performance of transgenic trees with altered lignification. Nature Biotechnology 20: 607-612.

Pilcher, C.P., Obrycki, J.J., Rice, M.E., Lewis, L.C. (1997): Preimaginal development, survival and field abundance of insect predators on transgenic Bacillus thuringiensis corn. Environmental Entomology, 26: 446-454.

Pilcher, C.D., Rice, M.E., Obrycki, J.J. (2005): Impact of transgenic Bacillus thuringiensis corn and crop phenology on five nontarget arthropods. Environmental Entomology: 1302-1316.

Pin, P., Zhang, W., Vogt, S., Dally, N., Büttner, B., Schulze-Buxloh, G., Jelly, N.S., Chia, T.Y.P., Mutasa-Göttgens, E., Dohm, J.C., Himmelbauer, H., Weisshaar, B., Kraus, J., Gielen, J.J.L., Lommel, M., Weyens, G., Wahl, B., Schechert, A., Nilsson, O., Jung, C., Kraft, T., Müller, A.E. (2012): The Role of a pseudo-response regulator gene in lifecycle adaptation and domestication of beet. Current Biology, DOI:10.1016/j.cub.2012.04.007.

Pineyro-Nelson, A., Van Heerwaarden, J., Perales, H.R., Serratos-Hernandez, J.A., Rangel, A., Hufford, M.B., Gepts, P., Garay-Arroyo, A., Rivera-Bustamante, R., Alvarez-Buylla, E.R. (2008): Transgenes in Mexican maize: molecular evidence and methodological considerations for GMO detection in landrace populations. Mol. Ecol. DOI: 10.1111/j.1365-294X.2008.03993.x.

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., Gouyon, P.H., Huet, S. (2007): Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. Journal of Applied Ecology. DOI:10.1111/j.1365-2664.2007.01358.x.

Pleasants, J.M., Hellmich, R.L., Dively, G.P., Sears, M.K., Stanley-Horn, D.E., Mattila, H.R., Foster, J.E., Clark, P.L., Jones, G.D. (2001): Corn pollen deposition on milk- weeds in or near cornfields. Proceedings of the National Academy of Sciences of the USA 98: 11919-11924. DOI:10.1073/ pnas.211287498.

Pleasants, J.M and Oberhauser, K.S. (2012): Milkweed loss in agricultural fields due to herbicide use: Effect on the Monarch Butterfly population. Insect Conservation and Diversity (online: 12 March, 2012).

Pline-Srnic, W. (2005): Technical performance of some commercial glyphosate-resistant crops. Pest Management Science 61, 225-234.

Poerschmann, J., Gathmann, A., Augustin, J., Langer, U., Górecki, T. (2005): Molecular composition of leaves and stems of genetically modified Bt and near-isogenic non-Bt maize characterization of lignin patterns. Journal of Environmental Quality 34: 1508-1518. DOI:10.2134/jeq2005.0070.

Pons, X., Lumbierres, B., López, C., Albajes, R. (2005): Abundance of non-target pests in transgenic Bt-maize: A farm scale study. European Journal of Entomology 102: 73-79.

Ponsard, S., Gutierrez, A.P., Mills, N.J. (2002): Effect of Bt-toxin (Cry1Ac) in transgenic cotton on the adult longevity of four heteropteran predators. Environmental Entomology 31: 1197-1205.

Porcar, M., Garcia-Robles, I., Dominguez-Escriba, L., Latorre, A. (2010): Effect of bacillus thuringiensis Cry1Ab and Cry3Aa endotoxins on predatory Coleoptera tested through artificial diet-incorporation bioassays. Bulletin of Entomological Research 100, 297-302. DOI:10.1017/ S0007485309990290.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E. (2010): Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution 25 (6), 354-353. DOI:10.1016/j.tree.2010.01.007.

Poulin, B., Lefebvre, G., Paz, L. (2010): Red flag for green spray: adverse trophic effects of Bti on breeding birds. Journal of Applied Ecology 2010, 47, 884-889. DOI:10.1111/j.1365-2664.2010. 01821.x.

Powell, J.R., Campbell, R.G., Dunfield, K.E., Gulden, R.H., Hart, M.M., Levy-Booth, D.J., Klironomos, J.N., Pauls, K.P., Swanton, C.J., Trevors, J.T., Antunes, P.M. (2009a): Effect of glyphosate on the tripartite symbiosis formed by Glomus intraradices, Bradyrhizobium japonicum, and genetically modified soybean. Applied Soil Ecology, 41: 128-136.

Powell, J.R., Gulden, R.H., Hart, M.M., Campbell, R.G., Levy-Booth, D.J., Dunfield, K.E., Pauls, K.P., Swanton, C.J., Trevors, J.T., Klironomos, J.N. (2007): Mycorrhizal and rhizobial colonization of genetically modified and conventional soybeans. Applied and Environmental Microbiology 73: 4365-4367. DOI:10.1128/AEM.00594-07.

Powell, J.R., Levy-Booth, D.J., Gulden, R.H., Asbil, W.L., Campbell, R.G., Dunfield, K.E., Hamill, A.S., Hart, M.M., Lerat, S., Nurse, R.E., Pauls, K.P., Sikkema, P.H., Swanton, C.J., Trevors, J.T., Klironomos, J.N. (2009b): Effects of genetically modified, herbicide-tolerant crops and their management on soil food web properties and crop litter decomposition. Journal of Applied Ecology, 46: 388-396.

Powles, S.B. (2008): Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Management Science 64: 360-365. DOI:10.1002/ps.1525.

Powles, S.B. (2010): Gene amplification delivers glyphosate-resistant weed evolution. PNAS Vol. 107(3), 955-956.

Powles, S.B., Lorraine-Colwill, D.F., Dellow, J.J., Preston, C. (1998): Evolved resistance to glyphosate in rigid ryegrass (Lolium rigidum) in Australia. Weed Science Journal 46: 604-607.

Powles, S.B., Yu, Q. (2010): Evolution in Action: Plants Resistant to Herbicides. Annual Review of Plant Biology 61, 317-47. DOI:10.1146/annurev-arplant-042809-112119.

Poza, M. de la, Pons, X., Farinós, G.P., López, C., Ortego, F., Eizaguirre, M., Castañera, P., Albajes, R. (2005): Impact of farm-scale Bt maize on abundance of predatory arthropods in Spain. Crop Protection 24: 677-684. DOI:10.1016/j.cropro.2004.12.003.

Prasifka, P.L., Hellmich, R.L., Prasifka, J.R., Lewis, L.C (2007): Effects of Cry1Ab-expressing corn anthers on the movement of monarch butterfly larvae. Environmental Entomology 36: 228-233.

Preston, C. (2010): Glyphosate-resistant rigid ryegrass in Australia. In: Nandula VK (Ed), Glyphosate Resistance in Crops and Weeds: History, Development, and Management, John Wiley & Sons, Inc., New York, pp 233-247.

Preston, C., Wakelin, A.M., Dolman, F.C., Bostamam, Y., Boutsalis, P. (2009): A decade of glyphosate-resistant Lolium around the world: mechanisms, genes, fitness and agronomic management. Weed Science 57, 435-441.

Priesnitz, K.U. (2010): Potential impact of Diabrotica resistant Bt-maize expressing Cry3Bb1 on ground beetles (Coleoptera: Carabidae). PhD Thesis, RWTH Aachen.

Prihoda, K.R. and Coats, J.R. (2008a): Aquatic fate and effects of Bacillus thuringiensis Cry3Bb1 protein: towards risk assessment. Environmental Toxicology and Chemistry 27, 793-798.

Prihoda, K.R. and Coats, J.R. (2008b): Fate of Bacillus thuringiensis (Bt) Cry3Bb1 protein in a soil microcosm. Chemosphere 73, 1102-1107.

Pritchard, J., Griffiths, B., Hunt, E.J. (2007): Can the plant mediated impacts on aphids of elevated CO2 and drought be predicted? Global Change Biology 13: 1616-1629. DOI:10.1111/j.1365-2486.2007.01401.x.

Prütz, G., Brink, A., Dettner, K. (2004): Transgenic insect-resistant corn affects the fourth trophic level: effects of Bacillus thuringiensis-corn on the facultative hyperparasitoid Tetrastichus howardi. Naturwissenschaften 91: 451-454. DOI:10.1007/s00114-004-0558-0.

Prütz, G. and Dettner, K. (2004): Effect of Bt corn leaf suspension on food consumption by Chilo partellus and life history parameters of its parasitoid Cotesia flavipes under laboratory conditions. Entomologia Experimentalis et Applicata 111: 179-187.

Qi, A., Perry, J.N., Pidgeon, J.D., Haylock, L.A., Brooks, D.R. (2008): Cost-efficacy in measuring farmland biodiversity – lessons from the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Annals of Applied Biology, 152: 93-101.

Qureshi, J.A., Buschman, L.L., Throne, J.E., Ramaswamy, S.B. (2005): Adult dispersal of Ostrinia nubilalis Hübner (Lepidoptera: Crambidae) and its implications for resistance management in Bt-maize. J. Appl. Entomol. 129: 281-292

Ramirez-Romero, R., Bernal, J.S., Chaufaux, J., Kaiser, L. (2007): Impact assessment of Bt-maize on a moth parasitoid, Cotesia marginiventris (Hymenoptera: Braconidae), via host exposure to purified Cry1Ab protein or Bt-plants. Crop Protection 26: 953-962. DOI:10.1016/j.cropro.2006.09.001.

Ramirez-Romero, R., Chaufaux, J., Pham-Delègue, M.H. (2005): Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee Apis mellifera, a comparative approach. Apidologie 36: 601-611. DOI:10.1051/apido:2005039.

Ramirez-Romero, R., Desneux, N., Decourtye, A., Chaffiol, A., Pham-Delègue, M.H. (2008): Does Cry1Ab protein affect learning performances of the honey bee Apis mellifera L. (Hymenoptera, Apidae). Ecotox. Environ. Safety 70: 327-333.

Ranjekar, P.K., Patankar, A., Gupta, V., Bhatnagar, R., Bentur J., Kumar, P.A. (2003): Genetic engineering of crop plants for insect resistance. Current Science 84: 321-329.

Raps, A., Kehr, J., Gugerli, P., Moar, W.J., Bigler, F., Hilbeck, A. (2001): Immunological analysis of phloem sap of Bacillus thuringiensis corn and of the nontarget herbivore Rhopalosiphum padi (Homoptera: Aphididae) for the presence of Cry 1Ab. Mol. Ecol. 10: 525-533.

Rasche, F., Hödl, V., Poll, C., Kandeler, E., Gerzabek, M., Elsas, J.D. van, Sessitsch, A. (2006b): Rhizosphere bacteria affected by transgenic potatoes with antibacterial activities compared with the effects of soil, wild-type potatoes, vegetation stage and pathogen exposure. FEMS Microbiology Ecology 56: 219-235. DOI:10.1111/j.1574-6941.2005.00027.x.

Rasche, F., Velvis, H., Zachow, C., Berg, G., Elsas, J.D. van, Sessitsch, A. (2006a): Impact of transgenic potatoes expressing anti-bacterial agents on bacterial endophytes is comparable with the effects of plant genotype, soil type and pathogen infection. Journal of Applied Ecology 43: 555-566. DOI: 10.1111/j.1365-2664.2006.01169.x.

Raubuch, M., Roose, K., Warnstorff, K., Wichern, F., Joergensen, R.G. (2007): Respiration pattern and microbial use of field-grown transgenic Bt-maize residues. Soil Biology & Biochemistry 39: 2380-2389. DOI:10.1016/j.soilbio.2007.04.012.

Rauschen, S. (2010): A case of “pseudo science”? A study claiming effects of the Cry1Ab protein on larvae of the two spottet ladybird is reminiscent of the case of the green lacewing. Transgenic Research, 13-16. DOI:10.1007/s11248-009-9301-0.

Rauschen, S., Eckert, J., Gathmann, A., Schuphan, I. (2004): Impact of growing Bt-maize on cicadas: Diversity; abundance and methods. IOBC wprs Bulletin 27: 137-142.

Rauschen, S., Schaarmschmidt, F., Gathmann, A. (2010a): Occurrence and field densities of Coleoptera in the maize herb layer: implications for environmental risk assessment of genetically modified Bt-maize. Transgenic Research 19, 727-744.

Rauschen, S., Schultheis, E., Hunfeld, H., Schaarschmidt, F., Schuphan, I., Eber, S. (2010b): Diabrotica-resistant Bt-maize DKc5143 event MON88017 has no impact on the field densities of the leafhopper Zyginidia scutellaris. Environmental Biosafety Research 9, 87-99.

Rauschen, S., Schultheis, E., Pagel-Wieder, S., Schuphan, I., Eber, S. (2009): Impact of Bt-corn MON88017 in comparison to three conventional lines on Trigonotylus caelestialium (Kirkaldy) (Heteroptera: Miridae) field densities. Transgenic Re-search 18, 203-214.

Rauschen, S. and Schuphan, I. (2006): Fate of the Cry1Ab protein from Bt-maize MON810 silage in biogas production facilities. J. Agric. Food Chem. 54: 879-883. DOI:10.1021/jf0525380.

Rauschen, S., Schuphan, I., Eber, S. (2008): Assessment of possible non-target impacts of the novel Bt-maize event MON88017 resistant against the Western corn rootworm Diabrotica virgifera virgifera (LeConte). In: Romeis J, Meissle M, Sanvido O (Eds), GMOs in Integrated Plant Production – Ecological impact of genetically modified organisms, IOBC wprs Bulletin 33, 93-99.

Rausell, C., Garcia-Robles, I., Sanchez, J., Munoz-Garay, C., Martniez-Ramirez, A.C., Real, M.D., Bravo, A. (2004a): Role of toxin activation on binding and pore formation activity of the Bacillus thuringiensis Cry3 toxins in membranes of Leptinotarsa decemlineata (Say). Biochim. Biophys. Act. Biomembr. 1660: 99-105.

Rausell, C., Pardo-López, L., Sánchez, J., Muñoz-Garay, C., Morera, C., Soberón, M., Bravo, A. (2004b): Unfolding events in the water soluble monomeric Cry1Ab toxin during transition to oligomeric pre-pore and membrane-inserted pore channel. The Journal of Biological Chemistry 279: 55168-55175. DOI:10.1074/jbc.M406279200.

Raybould, A. (2006): Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. Environ. Biosafety. Res., 5 (3), 119-125.

Raybould, A. (2010): Reducing uncertainty in regulatory decision-making for transgenic crops – More ecological research or clearer environmental risk assessment? GM Crops 1, 1-7.

Raybould, A. (2011): The bucket and the searchlight: formulating and testing risk hypotheses about the weediness and invasiveness potential of transgenic crops. Environmental Biosafety Research. DOI:10.1051/ebr/2011101.

Raybould; A., Caron-Lormier, G., Bohan, D.A. (2010): Derivation and Interpretation of Hazard Quotiens To Assess Ecological Risks from the Cultivation of Insect-Resistent Transgenic Crops. Journal of Agricultural and Food Chemistry. DOI:10.1021/jf042079.

Raybould, A.F., Gray, A.J. (1994): Will hybrids of genetically modified crops invade natural communities? Trends in Ecology and Evolution 9: 85-89.

Raybould, A., Stacey, D., Vlachos, D., Graser, G., Li, X., Joseph, R. (2007): Non-target organism risk assessment of MIR604 maize expressing mCry3A for control of corn rootworm. J. Appl. Entomol. (Journal of Applied Entomology) 131: 391-399. DOI:10.1111/j.1439-0418.2007.

Raybould, A., Vlachos, D. (2010): Non-target organism effects tests on Vip3A and their application to the ecological risk assessment for cultivation of MIR162 maize. Transgenic Research. DOI:10.1007/s11248-010-9442-1.

Raymond, B., Johnston, P.R., Nielsen-LeRoux, C., Lereclus, D., Crickmore, N. (2010): Bacillus thuringiensis: an impotent pathogen? Trends in Microbiology 18 (5), 189-194, DOI:10.1016/j.tim.-2010.02.006.

Ražanskienė, A., Rugienius, R., Gelvonauskiene, D., Vinskiene, J., Zalunskaite, I., Stankiene, J., Sasnauskas, A., Stanys, V. (2005): Construction of vectors carrying (BRV) DNA sequences in aim to induce RNA interference and virus resistance of black currants (Ribes nigrum L.) Fruit science, vol. 222, p. 103-109.

Ražanskienė, R., Stanienėm, G., Rugieniusm, R., Gelvonauskienėm, D., Zalunskaitem, I., Vinskienėm, J., Stanysm, V. (2006): Transformation of quince (Cydonia oblonga) with the rolB gene-based constructs under different promoters. Journal of Fruit and Ornamental Plant Research. Vol.14 (Suppl.1), 95-102.

Razze, J.M., Mason, C.E., Pizzolato, T.D. (2011): Feeding Behavior of Neonate Ostrinia nubilalis (Lepidoptera: Crambidae) on Cry1Ab Bt Corn: Implications for Resistance Management. Journal of Economic Entomology 104 (3), 806-813. DOI:10.1603/EC10287.

Reddy, K.N. and Whiting, K. (2000): Weed control and economic comparisons of glyphosate-resistant, sulfonylurea-tolerant, and conventional soybean (*Glycine max*) systems. Weed Technology 14, 204-211.

Reddy, K.N. and Zablotowicz, R.M. (2003): Glyphosate-resistant soybean response to various salts of gly-phosate and glyphosate accumulation in soybean nodules. Weed Science 51, 496-502.

Reidsma, P., Tekelenburg, T., van den Berg, M., Alkemade, R. (2006): Impacts of land-use change on biodiversity: As assessment of agricultural biodiversity in the European Union. Agriculture, Ecosystems and Environment 114: 86-102.

Reidsma, P. et al., (2006): Impacts of land-use change on biodiversity: As assessment of agricultural biodiversity in the European Union. Agriculture, Ecosystems and Environment 114: 86-102.

Reif, J., Vorisek, P., Stastny, K., Bejcek, V., Petr J. (2008): Agricultural intensification and farmland birds: new insights from a central European country. Ibis 150 (3): 596-605.

Reif, J. et al., (2008): Agricultural intensification and farmland birds: new insights from a central European country. Ibis 150 (3): 596-605.

Reus, J., Leendertse, P., Bockstaller, C., Fomsgaard, I., Gutsche, V., Lewis, K., Nilsson, C., Pussemier, L., Trevisan, M., van der Werf, H., Alfarroba, F., Blümel, S., Isart, J., McGrath, D., Seppälä, T. (2002): Comparison and evaluation of eight pesticide environmental risk indicators developed in Europe and recommendations for future use. Agriculture, Ecosystems & Environment 90, 177-187.

Reuter, H., Middelhoff, U., Graef, F., Verhoeven, R., Batz, T., Weis, M., Schmidt, G., Schroder, W., Breckling, B. (2010a): Information system for monitoring environmental impacts of genetically modified organisms. Environmental Science and Pollution Research. DOI:10.1007/s11356-010-0334-y.

Reuter, T., Alexander, T.W., Xu, W., Standford, K., McAllister, T.A. (2010b): Biodegradation of genetically modified seeds and plant tissues during composting. Journal of the Science and Food Agricultural 90, 650–657. DOI:10.1002/jsfa.3863.

Rhymer, J.M. and Simberloff, D. (1996): Extinction by hybridization and introgression. Annual Review of Ecology, Evolution, and Systematics 27: 83-109.

Richardsen, J.S., Perrin, C.J. (1994): Effects of bacterial insecticide Bacillus thuringiensis var kurstaki (Btk) on a stream benthic community. Canadian Journal of Fisheries and Aquatic Sciences 51: 1037-1045.

Ricroch, A., Berge, J.B., Kuntz, M. (2010): Is the German suspension of MON810 maize cultivation scientifically justified? Transgenic Research 19, 1-12. DOI:10.1007/s11248-009-9297-5.

Riddick, E.W., Dively, G., Barbosa, P. (1998): Effect of a seed-mix deployment of Cry3A-transgenic and nontransgenic potato on the abundance of Lebia grandis (Coleoptera: Carabidae) and Coleomegilla maculata (Coleoptera: Coccinellidae). Annals of the Entomologic Society of America 91: 647-653.

Riddick, E.W., Dively, G., Barbosa, P. (2000): Season-long abundance of generalist predators in transgenic versus nontransgenic potato fields. Journal of Entomological Science 35: 349-359.

Riesgo, L., Areal, F.J., Sanvido, O., Rodrígez-Cerezo, E. (2010): Distances needed to limit cross-fertilization between GM and conventional maize in Europe. Nature Biotechnology 28 (8), 780-782.

Robischon, M. (2006): Field trials with transgenic trees – state of the art and developments. In: Tree transgenesis: Recent Developments, Eds. Fladung, M. and Ewald, D.. Springer-Verlag Berlin Heidelberg p. 3-23.

Rodrígez-Cerezo, E., Czarnak-Kłos, M. (2010): 1. Maize crop production – Best Practice Documents for coexistence of genetically modified crops with conventional and organic farming. JRC Scientific and Technical Reports.

Rodrigo-Simón, A., Maagd, R.A. de, Avilla, C., Bakker, P.L., Moltoff, J., González-Zamora, J.E., Ferré, J. (2006): Lack of detrimental effects of Bacillus thuringiensis Cry toxins on the insect predator Chrysoperla carnea: a toxicological, histopathological, and biochemical analysis. Applied and Environmental Microbiology 72: 1595-1603. DOI:10.1128/AEM.72.2.1595–1603.2006.

Römbke, J., Jänsch, S., Meier, M., Hilbeck, A., Teichmann, H., Tappeser, B. (2010): General recommendations for soil ecotoxicological tests suitable for the Environmental Risk Assessment (ERA) of Genetically Modified Plants. Integrated Environmental Assessment and Management 6, 287-300. DOI:10.1897/IEAM.2009-043.1.

Romeis, J. (2006): Non-target risk assessment of GM crops and regulation. IOBC wprs Bulletin 29: 197-200.

Romeis, J., Álvarez-Alfageme, F., Bigler, F. (2012): Putative effects of Cry1Ab to larvae of *Adalia bipunctata* – reply to Hilbeck et al. (2012) Environmental Sciences Europe 2012, 24:18 DOI:10.1186/2190-4715-24-18.

Romeis, J., Babendreier, D., Wäckers, F.L. (2003): Consumption of snowdrop lectin (Galanthus nivalis agglutinin) causes direct effects on adult parasitic wasps. Oecologia 134: 528-536. DOI:10.1007/s00442-002-1144-9.

Romeis, J., Bartsch, D., Bigler, F., Candolfi, M.P., Gielkens, M.M.C., Hartley, S.E., Hellmich, R.L., Huesing, J.E., Jepson, P.C., Layton, R., Quemada, H., Raybould, A.F., Rose, R.I., Schiemann, J., Sears, M.K., Shelton, A.M., Sweet, J., Vaituzis, Z., Wolt, J.D. (2006a): Moving through the tiered and methodological framework for non-target arthropod risk assessment of transgenic insecticidal crops. Proceedings of the 9th International Symposium on the Biosafety of Genetically Modified Organisms, 24-29 September 2006, Jeju Island, South Korea.

Romeis, J., Bartsch, D., Bigler, F., Candolfi, M.P., Gielkens, M.M.C., Hartley, S.E., Hellmich, R.L., Huesing, J.E., Jepson, P.C., Layton, R., Quemada, H., Raybould, A., Rose, R.I., Schiemann, J., Sears, M.K., Shelton, A.M., Sweet, J., Vaituzis, Z., Wolt, J.D. (2008a): Assessment of risk of insect-resistant transgenic crops to non-target arthropods. Nature Biotechnology 26: 203-208. DOI:10.1038/nbt1381.

Romeis, J., Dutton, A., Bigler, F. (2004): Bacillus thuringiensis toxin (Cry1Ab) has no direct effect on larvae of the green lacewing Chrysoperla carnea. Journal of Insect Physiology 50: 175-183. DOI:10.1016/j.jinsphys.2003.11.004.

Romeis, J., Hellmich, R.L., Candolfi, M.P., Keri Carstens, K., De Schrijver, A., Angharad, M. R.Gatehouse, A.M.R., Herman, R.A., Huesing, J.E., McLean, M.A., Raybould, A., Shelton, A.M., Waggoner, A. (2010): Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. Transgenic Research. DOI:10.1007/s11248-010-9446-x.

Romeis, J., Meissle, M. (2010): Non-target risk assessment of Bt crops - Cry protein uptake by aphids. Journal of Applied Entomology, 1-6. DOI:10.1111/j.1439-0418.2010.01546.x.

Romeis, J., Meissle, M., Bigler, F. (2006b): Transgenic crops expressing Bacillus thuringiensis toxins and biological control. Nature Biotechnology 24: 63-71. DOI:10.1038/nbt1180.

Romeis, J., Meissle, M., Raybould, A., Hellmich, R.L. (2009): Impact of Insect-resistant Transgenic Crops on Aboveground Non-target Arthropods. In: Environmental Impact of Genetically Modified Crops (eds N. Ferry and A.M.R. Gatehouse), CAB International. p. 165-198.

Romeis, J., Van Driesche, R.G., Barratt, B.I.P., Bigler. F. (2008b): Insect-resistant transgenic crops and biological control. In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), Integration of insect-resistant genetically modified crops within IPM programs, Springer Science + Business Media BV, pp. 87-117.

Romeis, J., Waldburger, M., Streckeisen, Ph., Hogervorst, P.A.M., Keller, B., Winzeler, M., Bigler, F. (2007): Performance of transgenic spring wheat plants and effects on non-target organisms under glasshouse and semi-field conditions. J. Appl. Entomol. (Journal of Applied Entomology) 131: 593-602. DOI:10.1111/j.1439-0418.2007.01231.x.

Rose, R.I. (2007): White paper on tier-based testing for the effects of proteinaceous insecticidal plant-incorporated protectants on non-target invertebrates for regulatory risk assessment. USDA-APHIS and US Environmental Protection Agency, Washington DC, USA.

Rose, R. and Dively, G. (2007): Effects of insecticide-treated and lepidopteran-active Bt transgenic sweet corn on the abundance and diversity of arthropods. Environmental Entomology 36, H. 1254-1268.

Rose, R.I., Dively, G.P., Pettis, J. (2007): Effects of Bt corn pollen on honey bees: emphasis on protocol development. Apidologie 38. DOI:10.1051/apido:2007022.

Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Whiles, M.R., Evans-White, M., Chambers, C., Griffiths, N.A., Pokelsek, J., Stephen, M.L. (2007): Toxins in transgenic crop byproducts may affect headwater stream ecosystems. Proceedings of the National Academy of Sciences of the USA 104: 16204-16208. DOI:10.1073/pnas.0707177104.

Rüdelsheim, P.L.J., Smets, G. (2010): Anticipating the Environmental Risk Assessment of crops modified to enhance or preserve yield. Perseus – Biosefety and Biotechnology Regulatory Services.

Rühl, G., Hommel, B., Hüsken, A., Mastel, K., Schiemann, J., Wehling, P., Langhof, M. (2011): Coexistence in Maize: Effect on Pollen-Mediated Gene Flow by Conventional Maize Border Rows Edging Genetically Modified Maize Fields. Crop Science Vol. 51, 1-9. DOI:10.2135/cropsci-2010.08.0466.

Ruttink, T; Morisset, D., van Droogenbroeck, B., Lavrac, N., van den Eede, G., Zel, J., de Loose, M. (2010): Knowledge-technology-based discovery of unauthorized genetically modified organisms. Analytical and Bioanalytical Chemistry 396, 1951–1959. DOI:10.1007/s00216-009-3218-6.

Ryle, G.J.A., Powell, C.E. (1992): The influence of elevated CO2 and temperature on biomass production of continuously defoliated white clover. Plant Cell Environment 15: 593-599.

Saeglitz, C. and Bartsch, D. (2002): Plant gene flow consequences. AgBiotechNet 4: ABN 084.

Saeglitz, C., Gathmann, A., Priesnitz, K.U., Schuphan, I., Bartsch, D. (2006): Monitoring the Cry1Ab susceptibility of European Corn Borer (Ostrinia nubilalis Hbn.) in Germany. Journal of Economic Entomology 99: 1768-1773.

Sanahuja, G., Banakar, R., Twyman, R.M., Capell, T., Christou, P. (2011): Bacillus thuriengiensis: a century of research, development and commercial applications. Plant Biotechnology Journal 9, 283-300. DOI:10.1111/j.1467-7652.2011.00595.x.

Sanchis, V. (2010): From microbial sprays to insect-resistant transgenic plants: history of the biospesticide Bacillus thuringiensis. A review. Agronomy for Sustainable Development. DOI:10.1051-/agro/2010027.

Sanders, C.J., Pell, J.K., Poppy, G.M., Raybould, A., Garcia-Alonso, M., Schuler, T.H. (2007): Host-plant mediated effects of transgenic maize on the insect parasitoid Campoletis sonorensis (Hymenoptera: Ichneumonidae). Biological Control 40: 362-369. DOI:10.1016/j.biocontrol.-2006.12.010.

Sanogo, S., Yang, X.B., Lundeen, P. (2001): Field response of glyphosate-tolerant soybean to herbicides and sudden death syndrome. Plant Disease 85: 773-779.

Santos, M.N., Nogueira, P.M., Dias, F.B.S., Valle, D., Moreira, L.A. (2010): Fitness aspects of transgenic Aedes fluviatilis mosquitoes expressing a Plasmodium-blocking molecule. Transgenic Research. DOI:10.1007/s11248-010-9375-8.

Sanvido, O., Romeis, J., Bigler, F. (2007): Ecological impacts of genetically modified crops: Ten years of field research and commercial cultivation. Adv Biochem Engin/Biotechnol 107: 235-278. DOI:10.1007/10_2007_048.

Sanvido, O., Romeis, J., Bigler, F., (2008a): An approach for post-market monitoring of potential environmental effects of Bt-maize expressing Cry1Ab on natural enemies. Journal of Applied Entomology, 133:236-248.

Sanvido, O., Romeis, J., Bigler, F. (2011a): Environmental change challenges decision-making during post-market environmental monitoring of transgenic crops. Transgenic Research. DOI:10.1007/s11248-011-9524-8.

Sanvido, O., Romeis, J., Gathmann, A., Gielkens, M., Raybould, A., Bigler, F. (2011 c): Evaluating environmental risks of genetically modified crops – ecological harm criteria for regulatory decision-making. Environment & Science Policy, DOI:10.1016/j.envsci.2011.08.006.

Sanvido, O., Schrijver de, Adinda; Devos, Y., Bartsch, D. (2011b): Post Market Environmental Monitoring of Genetically Modified Herbicide Tolerant Crops (Working group report from the 4th International Workshop PMEM of Genetically Modified Plants, Quedlinburg, Germany 2010): Journal für Kulturpflanzen 63 (7), 211-216.

Sanvido, O., Widmer, F., Winzeler, M., Streit, B., Szerencsits, E., Bigler, F. (2008b): Definition and feasibility of isolation distances for transgenic maize cultivation. Transgenic Research 17:317-335.

Saxena, D., Flores, S., Stotzky, G. (2002a): Vertical movement in soil of insecticidal Cry1Ab protein from Bacillus thuringiensis. Soil Biology & Biochemistry 34: 111-120.

Saxena, D., Flores, S., Stotzky, G. (2002b): Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biology & Biochemistry 34: 133-137. DOI:10.1016/S0038-0717(01)00161-4.

Saxena, D. and Stotzky, G. (2001a): Bacillus thuringiensis (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biology & Biochemistry 33: 1225-1230.

Saxena, D. and Stotzky, G. (2001b): Bt corn has a higher lignin content than non-Bt corn. American Journal of Botany 88: 1704-1706.

Saxena, D. and Stotzky, G. (2001c): Bt toxin uptake from soil by plants. Nature Biotechnology 19: 199.

Saxena, D. and Stotzky, G. (2002): Bt toxin is not taken up from soil or hydroponic culture by corn, carrot, radish or turnip. Plant and soil, 239: 165-172.

SCF (2001): Guidance on submissions for food additive evaluations by the scientific committe on food. http://ec.europa.eu/food/fs/sc/scf/out98_en.pdf

Scherm, H., Sutherst, R.W., Harrington, R., Ingram, J.S.I. (2000): Global networking for assessment of impact of global change on plant pests: Climate change: Potential scenarios for plant diseases and air pollutants. Environmental Pollution 108: 317-395.

Schmidt, J.E.U., Braun, C.U., L'Abate, C., Whitehouse, L.P., Hilbeck, A. (2004): Studies on effects of Bacillus thuringiensis-toxins from transgenic insect-resistant plants on predaceous lady beetles (Coleoptera: Coccinellidae). [Original Title: Untersuchungen zu Effekten von Bacillus thuringiensis-Toxinen aus transgenen insektenresistenten Pflanzen auf räuberische Marienkäfer (Coleoptera: Coccinellidae).]. Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie 14, H. 419-422.

Schmidt, J.E.U., Braun, C.U., Whitehouse, L.P., Hilbeck, A. (2009): Effects of activated Bt transgene products (Cry1Ab, Cry3Bb) on immature stages of the ladybird Adalia bipunctata in laboratory ecotoxicity testing. Archives of Environmental Contamination and Toxicology, 56: 221-228.

Schmidt, K., Wilhelm, R., Schmidtke, J., Beißner, L., Mönkemeyer, W., Böttinger, P., Sweet, J., Schiemann, J. (2008): Farm questionnaires for monitoring genetically modified crops: a case study using GM maize. Environ. Biosafety Res. 7: 163-179.

Schnepf, E., Crickmore, N., Rie, J. van, Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R., Dean, D.H. (1998): Bacillus thuringiensis and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews 62: 775-806.

Schrader, S., Münchenberg, T., Baumgarte, S., Tebbe, C.C. (2008): Earthworms of different functional groups affect the fate of the Bt-toxin Cry1Ab from transgenic maize in soil. Eur. J. Soil Biol. 44: 283-289.

Schubert, G. (2010): Zwanzig Jahre Gentechnikgesetz – eine Erfolgsgeschichte? NVwZ 14/2010.

Schuler, T.H. (2006): New Bt toxins in the EU GMO application process: A review of published research into impacts on non-target insects and ecosystem services. UK Department for Environment, Food and Rural Affairs (Eds); DEFRA report53. http://www.defra.gov.uk/environment/gm/research/pdf/epg-ts1.pdf

Schuler, T.H., Potting, R.P.J., Denholm, I., Clark, S.J., Clark, A.J., Stewart, C.N., Poppy, G.M. (2003): Tritrophic choice experiments with Bt plants, the diamondback moth (Plutella xylostella) and the parasitoid Cotesia plutellae. Transgenic Research 12: 351-361. DOI:10.1023/A:1023342027192.

Schuphan, I. (2006): Protecting the benefits of Bt-toxins from insect resistance development by monitoring and management. RWTH Aachen (Eds). http://www.bio5.rwth-aachen.de/german/downloads/EU-Review.pdf

Schütte, G., Mertens, M. (2010): Potential effects of the introduction of a sugar beet variety resistant to glyphosate on agricultural practise and on the environment. BfN-Skripten 277, 61 p.

Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattila, H.R., Siegfried, B.D., Dively, G.P. (2001): Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. Proceedings of the National Academy of Sciences of the USA 98: 11937-11942. DOI:10.1073/pnas.211329998.

Sederoff, R. (2007): Regulatory science in forest biotechnology, Tree Genetics & Genomes, 3(2), 71-74.

Seitz, H., Züghart, W., Finck, M., Beismann, H., Bernhorn, F., Eikmann, T. (2010): Standardisierung von Methoden für ein Monitoring der Umweltwirkungen gentechnisch veränderter Pflanzen, Erarbeitung von VDI-Richtlinien. Bundesamt für Naturschutz.

Shaner, D.L., Lindenmeyer, R.B., Ostlie, M.H. (2011): What have the mechanisms of resistance to glyphosate taught us? Pest Management Science, DOI:10.1002/ps.2261.

Shaw, D.R., Givens, W.A., Farno, L.A., Gerard, P.D., Jordan, D., Johnson, W.G., Weller, S.C., Young, B.G., Wilson, R.G., Owen, M.D.K. (2009): Using a grower survey to assess the benefits and challenges of glyphosate-resistant cropping systems for weed management in US corn, cotton, and soybean. Weed Technology 23, 134-149.

Shaw, D.R., Owen, M.D.K., Dixon, P.M., Weller, S.C., Young, B.G., Wilson, R.G., Jordan, D.L., (2011): Benchmark study on glyphosate-resistant cropping systems in the United States. Part 1: Introduction to 2006-2008. Pest Management Science. DOI:10.1002/ps.2160.

Shen, J.L., Zhou, W.J., Wu, Y.D., Lin, X.W., Zhu, X.F. (1998): Early resistance of Helicoverpa to Bacillus thuringiensis and its relation to the effect of transgenic cotton lines expressing Bt toxin on the insect. Acta Entomologica Sinica 41: 8-14.

Siegfried, B.D., Vaughn, T.T., Spencer, T. (2005): Baseline susceptibility of Western Corn Rootworm (Coleoptera: Crysomelidae) to Cry3Bb1 Bacillus thuringiensis toxin. Journal of Economic Entomology 98: 1320-1324.

Simard, M.J., Légère, A., Warwick, S.I. (2006): Transgenic Brassica napus fields and Brassica rapa weeds in Quebec: Sympatry and weed-crop in situ hybridisation. Canadian Journal of Botany 84: 1842-1851. DOI:10.1139/B06-135.

Simpson, E., McRoberts, N., Sweet J. (2006): Out-crossing between genetically modified herbicide tolerant and other winter oilseed rape cultivars. Plant Genetic Resources, Volume 4, Number 2, pp. 96-107(12).

Sims, S.R. and Holden, L.R. (1996): Insect bioassay for determining soil degradation of Bacillus thuringiensis subsp. kurstaki CryIA(b) protein in corn tissues. Environmental Entomology 25: 659-664.

Sissener, N.H., Johannessen, L.E., Hevroy, E.M., Wiik-Nielsen, C.R., Berdal, K.G., Nordgreen, A., Hemre, G.I. (2010): Zebrafish (Danio rerio) as a model for investigating the safety of GM feed ingredients (soya and maize); performance, stress response and uptake of dietary DNA sequences. British Journal of Nutrition 103: 3-15. DOI:10.1017/S0007114509991401.

Sisterson, M.S., Biggs, R.W., Olson, C., Carrière, Y., Dennehy, T.J., Tabashnik, B.E. (2004): Arthropod abundance and diversity in Bt and non-Bt cotton fields. Environmental Entomology 33: 921-929.

Sisterson, M.S., Carrière, Y., Dennehy, T.J., Tabashnik, B.E. (2007): Nontarget effects of transgenic insecticidal crops: Implications of source-sink population dynamics. Environmental Entomology 36: 121-127.

Slavov, G.T., Di Fazio, S., Strauss, S.H. (2004): Gene flow in forest tress: Gene migration patterns and landscape modelling of transgene dispersisal in hybrid poplar. In: Den Nijs, H., Bartsch, D., and Sweet, J. (eds.) Introgression from Genetically Modified Plants into wild relatives and its consequences, CABI Wallingford, 89-106.

Smit, E., Bakker, P.A.H.M., Bergmans, H., Bloem, J., Griffiths, B.S., Rutgers, M., Sanvido, O., Singh, B.K., van Veen, H., Wilhelm, R., Glandorf, D.C.M. (2011): General Surveillance of the soil ecosystem: An approach to monitoring unexpected adverse effects of GMO's. Ecological Indicators 14, 107-113.

Smyth, S.J., Gusat, M., Belcher, K., Phillips, P.W.B., Castle, D. (2011): Environmental impacts from herbicide tolerant canola production in Western Canada. Agricultural Systems 104: 403-410. DOI:10.101016/j.agsy.2011.01.004.

Snow, A.A. (2002a): Possible phenotypic effects of genetically modified pathways on gene flow from field tests. Presented at: Criteria for field testing of plants with engineered regulatory, metabolic, and signalling pathways. Cited from Owen (2005).

Snow, A.A. (2002b): Transgenic crops – why gene flow matters. Nature Biotechnology 20: 542.

Snow, A.A., Andersen, B., Jørgensen, R.B. (1999): Costs of transgenic herbicide resistance introgressed from Brassica napus into weedy B. rapa. Molecular Ecology 8: 605-615. DOI:10.1046/j.1365-294x.1999.00596.x.

Snow, A.A., Andow, D.A., Gepts, P., Hallerman, E.M., Power, A., Tiedje, J.M., Wolfenbarger, L.L. (2005): ESA Report – Genetically engineered organisms and the environment: Current status and recommendations. Ecological Applications 15: 377-404.

Snow, A.A., Campbell, L.G. (2011): Long-term Introgression of Crop Alleles in Weed Populations. ISB News Report Agricultural and Environmental Biotechnology.

Snow, A.A., Culley, T.M., Campbell, L.G., Sweeney, P.M., Hegde, S.G., Ellstrand, N.C. (2010): Long-term persistence of crop alleles in weedy populations of wild radish (Raphanus raphanistrum): New Phytologist 186: 537-548. DOI:10.1111/j.1469-8137.2009.03172.x.

Snow, A.A., Pilson, D., Rieseberg, L.H., Paulsen, M.J., Pleskac, N., Reagon, M.R., Wolf, D.E., Selbo, S.M. (2003): A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. Ecological Applications 13: 279-286. DOI:10.1890/1051-0761(2003)013[0279:ABTRHA]2.0.CO;2.

Snow, A.A., Uthus, K.L., Culley, T.M. (2001): Fitness of hybrids between weedy and cultivated radish: Implications for weed evolution. Ecological Applications 11: 934-943.

Soberón, M., Gill, S.S., Bravo, A. (2009): Signaling versus punching hole: how do Bacillus thuringiensis toxins kill insecto midgut cells? Cellular and Molecular Life Science, 66: 1337-1359.

Soberón, M., Pardo-Lopez, L., Lóopez, I., Gómez, I., Tabashnik, B.E., Bravo, A. (2007): Engineering modified Bt toxins to counter insect resistance. Science, 318: 1640-1642.

Soukup, J., Holec, J., Jursík, M., Hamouzová, K. (2011): Environmental and agronomic monitoring of adverse effects due cultivation of genetically modified herbicide tolerant crops. Journal für Verbraucherschutz und Lebensmittelsicherheit. DOI:10.1007/s0003-011-0682-7.

Soukup, J., Nováková, K., Hamouz, P., Námestek, J. (2006): Ecology of silky bent grass (Apera spica-venti (L.) Beauv.), its importance and control in the Czech Republic. Journal of Plant Diseases and Protection 113: 73-80.

Sparrow, P.A.C. (2010): GM Risk Assessment. Molecular Biotechnology, 44, 267–275. DOI:10.1007/s12033-009-9237-x.

Squire, G.R., Breckling, B., Dietz Pfeilstetter, A., Jorgensen, R.B., Lecomte, J., Pivard, S., Reuter, H., Young, M.W. (2010): Status of feral oilseed rape in Europe: its minor role as a GM impurity and its potential as a reservoir of transgene persistence. Environmental Science and Pollution Research. DOI:10.1007/s11356-010-0376-1.

Squire, G.R., Breckling, B., Dietz Pfeilstetter, A., Jorgensen, R.B., Lecomte, J., Pivard, S., Reuter, H., Young, M.W. (2011): Status of feral oilseed rape in Europe: its minor role as a GM impurity and its potential as a reservoir of transgene persistence. Environmental Science and Pollution Research 18, 111-115. DOI:10.1007/s11356-010-0376-1.

Squire, G.R., Brooks, D.R., Bohan, D.A., Champion, G.T., Daniels, R.E., Haughton, A.J., Hawes, C., Heard, M.S., Hill, M.O., May, M.J., Osborne, J.L., Perry, J.N., Roy, D.B., Woiwod, I.P., Firbank, L.G. (2003): On the rationale and interpretation of the Farm Scale Evaluation of genetically modified herbicide-tolerant crops. Philosophical Transactions of the Royal Society, B 358: 1779-1799. DOI:10.1098/rstb.2003.1403.

Squire, G.R., Hawes, C., Begg, G.S., Young, M.W. (2009): Cumulative impact of GM herbicide-tolerant cropping on arable plants assessed through species-based and functional taxonomies. Environmental Science and Pollution Research, 16: 85-94.

Srivastava, N., Gupta, V. K., Pati, R., Gaur, R. K. (2011): Genetically modified crops: an overview. Biotechnology 10, 136-148.

SSC (2000): First Report of the Scientific Steering Committee on the Harmonisation of Risk Assessment Procedures. European Commission, Health & Consumer Protection Directorate-General. http://europa.eu.int/comm/food/fs/sc/ssc/out83_en.pdf

Stanley-Horn, D.E., Dively, G.P., Hellmich, R.L., Mattila, H.R., Sears, M.K., Rose, R., Jesse, L.C.H., Losey, J.E., Obrycki, J.J., Lewis, L. (2001): Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. Proceedings of the National Academy of Sciences of the USA 98: 11931-11936. DOI:10.1073/pnas.211277798.

Stanton, M.L., Snow, A.A., Handel, S.N. (1986): Floral evolution: Attractiveness to pollinators increases male fitness. Science 232: 1625-1627. DOI:10.1126/science.232.4758.1625.

Stein, A.J., Rodrígez-Cerezo, E. (2010): International trade and the global pipeline of new GM crops. Nature Biotechnology 28 (1), 23-25.

Stewart, C.N., All, J.N., Raymer, P.L., Ramachandran, S. (1997): Increased fitness of transgenic insecticidal rapeseed. Molecular Ecology 6: 773-779. DOI:10.1046/j.1365-294X.1997.00239.x.

Stodola, T.J., Andow, D.A., Hyden, A.R., Hinton, J.L., Roark, J.J., Bushman, L.L., Porter, P., Cronholm, G.B. (2006): Frequency of resistance to Bacillus thuringiensis toxin Cry1Ab in Southern United States corn belt population of European corn borer (Lepidoptera: Crambidae). Journal of Economic Entomology 99: 502-507.

Stokstad, E. (2011): Can biotech and organic farmers get along? Science 332, 166-169

Storer, N.P., Babcock, J., Schlenz, M., Meade, T., Thompson, G.D., Bing, J.W; Huckaba, R.M. (2010): Discovery and Characterization of Field Resistance to Bt Maize: Spodoptera frugiperda (Lepidoptera: Noctuidae) in Puerto Rico. DowAgro Sciences, 131-138.

Storer, N.P., Kubiszak, M.E., King, J.E., Thompson, G.D., Santos, A.S. (2012): Status of resistance to Bt maize in *Spodoptera frugiperda*: Lessons from Puerto Rico, Journal of Invertebrate Pathology. http://dx.doi.org/10.1016/j.jip.2012.04.007

Strandberg, B. and Pedersen, M.B. (2002): Biodiversity in glyphosate tolerant fodder beet fields– timing of herbicide application. NERI Technical Report no. 410. Silkeborg, Denmark: National Environmental Research Institute. http://www2.dmu.dk/1_viden/2_Publikationer/3_fagrapporter/rapporter/FR410.pdf

Strandberg, B., Pedersen, M.B., Elmegaard, N. (2005): Weed and arthropod populations in conventional and genetically modified herbicide tolerant fodder beet fields. Agriculture, Ecosystems and Environment 105: 243-253. DOI:10.1016/j.agee.2004.03.005.

Strauss, S.H., Brunner, A.M., Busov, V.B., Ma, C., Meilan, R. (2004): Ten lessons from 15 years of transgenic Populus research. Forestry 2004 77(5):455-465; DOI:10.1093/forestry/77.5.455.

Strauss, S.Y., Rudgers, J.A., Lau, J.A., Irwin, R.E. (2002): Direct and ecological costs of resistance to herbivory. Trends in Ecology and Evolution 17: 278-285. DOI:10.1016/S0169-5347(02)02483-7.

Streloke, M. (2011): Risk assessment and management of herbicides: obligations of the new EU regulations. Journal of Consumer Protection and Food Safety 6, 55-59.

Sukopp, U., Pohl, M., Driessen, S., Bartsch, D. (2005): Feral beets – with help from the maritime wild? In: Gressel, J. (Ed); Crop ferality and volunteerism: 45-57. CRC Press.

Sustar-Vozlic, J., Rostohar, K., Blejec, A., Kozjak, P., Cergan, Z., Meglic, V. (2010): Development of sampling approaches for the determination of the presence of genetically modified organisms at the field level. Springer Link (396), 2031-2041.

Svobodová, Z., Habuštova, O., Hussein, H.M., Půža, V., Sehnal, F. (2012): Impact of genetically modified maize expressing Cry3Bb1 on non-target arthropods: first year results of a field study. In: Romeis J, Meissle M, Álvarez-Alfageme (Eds), GMOs in Integrated Plant Production, IOBC wprs Bulletin 73, 105-117.

Sweet, J.B. (2003): Pollen dispersal and cross pollination. Proceedings of the First European Conference on the Co-existence of Genetically Modified crops with Conventional and Organic crops, Denmark. Danish Institute for Agricultural Sciences, 21-32.

Sweet, J. (2009): The 10th International Symposium on the Biosafety of Genetically Modified Organisms (ISBGMO), Wellington, New Zealand, November 2008. Environmental Biosafety Research.

Sweet, J., Bartsch, D. (2011): Guidance on risk assessment of herbicide tolerant GM plantsby the European Food Safety Authority. Journal für Verbraucherschutz und Lebensmittelsicherheit. DOI:10.1007/s00003-011-0686-3.

Sweet, J.B. and Lutman, P.J.W. (2006): A commentary on the BRIGHT programme on herbicide tolerant crops and the implications of the BRIGHT and Farm Scale Evaluation programmes for the development of herbicide tolerant crops in Europe. Outlooks on Pest Management, December 2006, 249-254.

Sweet, J. B., Simpson E., Law., J, Lutman, P.J., Berry, K., Payne, R., Champion, G., May, M. Walker, K., Wightman, P. and Lainsbury, M. (2004): Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance in winter oilseed rape and sugar beet (BRIGHT Project). HGCA Project Report No 353, 265 pp. (www.hgca.com)

Szekeres, D., Kádár, F., Dorner, Z. (2008): Ground beetle (Coleoptera: Carabidae) in transgenic herbicide tolerant maize hybrids: impact of the transgenic crops or the weed control practice? In: Romeis J, Meissle M, Sanvido O (Eds), GMOs in Integrated Plant Production – Ecological impact of genetically modified organisms, IOBC wprs Bulletin 33, 105-110.

Tabashnik, B.E. (1994): Evolution of resistance to Bacillus thuringiensis. Annual Review of Entomology 39, 47-79.

Tabashnik, B.E. (2008): Delaying insect resistance to transgenic crops. Proc. Natl. Acad. Sci.USA 105: 19029-19030.

Tabashnik, B.E. (2010): Communal Benefits of Transgenic Corn. Science Vol. 330.

Tabashnik, B.E., Carrière, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M., Zhao, J.Z. (2003): Insect resistance to transgenic Bt crops: lessons from the laboratory and field. Journal of Economic Entomology 96: 1031-1038.

Tabashnik, B.E., Cushing, N.L., Finson, N., Johnson, M.W. (1990): Field development of resistance to Bacillus thuringiensis in diamondback moth (Lepidoptera: Plutellidae). Journal of Economic Entomology 83: 1671-1676.

Tabashnik, B.E., Gassmann, A.J., Crowder, D.W., Carrière, Y. (2008a): Insect resistance to Bt crops: evidence versus theory? Nat. Biotechnol. 26: 199-202.

Tabashnik, B.E., Gassmann, A.J., Crowder, D.W., Carrière, Y. (2008b): Reply to Field-evolved resistance to Bt toxins. Nat. Biotechnol. 26: 1074-1076.

Tabashnik, B.E., Morin, S., Unnithan, G.C., Yelich, A.J., Ellers-Kirk,C., Harpold, V.S., Sisterson, M.S., Ellsworth, P.C., Dennehy, T.J., Antilla, L., Liesner, L., Whitlow, M., Staten, R.T., Fabrick, J.A., Li, X., Carrière, Y., (2012): Sustained susceptibility of pink bollworm to Bt cotton in the United States. GM Crops and Food: Biotechnology in Agriculture and the Food Chain 3:3, 1-7.

Tabashnik, B.E., Sisterson, M.S., Ellsworth P.C., Dennehy, T., Antilla, L., Liesner, L., Whitlow, M., Staten, R.T., Fabrick, J.A., Unnithan, G.C., Yelich, A.J., Ellers-Kirk, C., Harpold, V.S., Li, X., Carriere, Y. (2010): Suppressing resistance to Bt cotton with sterile insect releases. Nature Biotechnology 28 (12). DOI:10.1038/nbt.1704.

Tabashnik, B.E., Van Rensburg, J.B.J., Carrière, Y. (2009): Field-evolved insect resistance to Bt crops: definition, theory and data. Journal of Economic Entomology 102, 2011-2025.

Tan, F., Wang, J., Feng, Y., Chi, G., Kong, H., Qiu, H., Wei, S. (2010): Bt corn plants and their straw have no apparent impact on soil microbial communities. Plant Soil (329), 349-364.

Tank, J.L., Rosi-Marshall, E., Royer, T.V., Whiles, M.R., Griffiths, N.A., Frauendorf, T.C., Treering, D.J. (2010): Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape. PNAS Early Edition, 1-6.

Tapp, H. and Stotzky, G. (1998): Persistence of the insecticidal toxin from Bacillus thuringiensis subsp. kurstaki in soil. Soil Biology & Biochemistry 30: 471-476. DOI:10.1016/S0038-0717(97)00148-X.

Taub, D.R., Miller, B., Allan, H. (2008): Effects of elevated CO2 on the protein concentration of food crops: a meta-analysis. Global Change Biology. DOI:10.1111/j.1365-2486.2007.01511.x.

Tepfer, D., Garcia-Gonzalez, R., Mansouri, H., Seruga, M., Message, B., Leach, F., Perica, M. (2003): Homology-dependent DNA transfer from plants to a soil bacterium under laboratory conditions: implications in evolution and horizontal gene transfer. Transgenic Research 12: 425-437.

Tepfer, M. (2002): Risk assessment of virus-resistant transgenic crops. Annual Review of Phytopathology 40: 467-491.

Tharp, B.E., Kells, J.J., Bauman, T.T., Harvey, R.G., Johnson, W.G., Loux, M.M., Martin, A.R., Maxwell, D.J., Owen, M.D.K., Regehr, D.L., Warnke, J.E., Wilson, R.G., Wrage, L.J., Young, B.G., Dalley, C.D. (2004): Assessment of weed control strategies for corn in the North-Central United States. Weed Technology, 18: 203-210.

Then, C. (2010): Agro-Gentechnik: Gentechnisch veränderter Mais fördert Ausbreitung von Schädlingen, Report 1-23.

Thieme, T. (2010): Impact of Roundup Ready® maize production systems on NTO's 'North Europe'. http://www.slideshare.net/smamu/t-thieme

Thomas, C.M. and Nielsen, K. M. (2005): Mechanisms and barriers to horizontal gene transfer between bacteria. Nature Reviews Microbiol. 3, 711-721.

Tian, J., Collins, H.L., Romeis, J., Naranjo, S.E., Hellmich, R.L., Shelton, A.M. (2012): Using field-evolved resistance to Cry1F maize in a lepidopteran pest to demonstrate no adverse effects of Cry1F on one of its major predators. Transgenic Research online first DOI 10.1007/s11248-012-9604-4.

Tiimonen, H., Aronen, T., Laakso, T., Saranpää, P., Chiang, V., Ylioja, T., Roininen, H., Häggman, H. (2005): Does lignin modification affect feeding preference and growth performance of insect herbivores in transgenic silver birch (Betula pendula Roth)? Planta 222, 699-708.

Tiimonen, H., Häggman, H., Tsai, C-J., Chiang, V., Aronen, T. (2007): The tissue-specific expression of PtCOMT promoter in Betula pendula Roth during the growing season and as a response to mechanical bending and wounding. Plant Cell Reports 26, 1205-1214.

Toschki, A., Hothorn, L.A., Roß-Nickoll, M. (2007): Effects of cultivation of genetically modified Bt maize on epigeic arthropods (Araneae; Carabidae). Environmental Entomology 36: 966-980.

Tothova, T., Sobekova, A., Holovska, K., Legath, J., Pristas, P., Javorsky, P. (2010): Natural glufosinate resistance of soil microorganisms and GMO safety. Central European Journal of Biology 5 (5), 656-663. DOI:10.2478/s11535-010-0042-0.

Traore, S.B., Carlson, R.E., Pilcher, C.D., Rice, M.E. (2000): Bt and non-Bt maize growth and development as affected by temperature and drought stress. Agronomy Journal 92: 1027-1035.

Trigo, E.J., Cap, E.J. (2003): The impact of the introduction of transgenic crops in Argentinean agriculture. AgBioForum 6: 87-94.

Turlings, T.C.J. and Ton, J. (2006): Exploiting scents of distress: the prospect of manipulating herbivore-induced plant odours to enhance the control of agricultural pests. Current Opinion in Plant Biology 9: 421-427. DOI:10.1016/j.pbi.2006.05.010.

Turrini, A., Sbrana, C., Nuti, M.P., Pietrangeli, B., Giovannetti, M. (2004): Development of a model system to assess the impact of genetically modified corn and aubergine plants on arbuscular mycorrhizal fungi. Plant and Soil 266: 69-75. DOI:10.1007/s11104-005-4892-6.

Tyutyunov, Y., Zhadanovskaya, E., Bourguet, D., Arditi, R. (2008): Landscape refuges delay resistance of the European corn borer to Bt-maize: a demo-genetic dynamic model. Theor. Popul. Biol. 74: 138-146.

USDA (2011): Glyphosate-Tolerant H7-1 Sugar Beets: Request for Nonregulated Status. Draft Environmental Impact Statement, October 2011. http://www.aphis.usda.gov/brs/aphisdocs/03_32301p_deis.pdf

Vacher, C., Weis, A.E., Hermann, D., Kossler, T., Young, C., Hochberg, M.E. (2004): Impact of ecological factors on the initial invasion of Bt transgenes into will populations of birdseed rape (Brassica rapa). Theoretical and Applied Genetics 109: 806-814. DOI:10.1007/s00122-004-1696-7.

Vachon, V., Laprade, R., Schwartz, J-L., (2012): Current models of the mode of action of *Bacillus thuringiensis* insecticidal crystal proteins: a critical review, Journal of Invertebrate Pathology (2012), DOI: http://dx.doi.org/10.1016/j.jip.2012.05.001

Van de Wiel, C.C.M., Flavell, A., Syed, N., Antonise, R., Voort, J.R. van der, Linden, C.G. van der (2004): Analysis of gene flow in the lettuce crop-weed complex In: Introgression from genetically modified plants into wild relatives / Nijs, J. den, Bartsch, D., Sweet, J., – Wallingford : CABI Publishing.

Van der Werf, H.M.C. (1996): Assessing the impact of pesticides on the environment. Agriculture, Ecosystems & Environment 60, 81-96.

Van Rensburg, J.B.J. (2007): First report of field resistance by the stem borer, Busseola fusca (Fuller) to Bt-transgenic maize. South African J. Plant Soil 24: 147-151.

Vercesi, M.L., Krogh, P.H., Holmstrup, M. (2006): Can *Bacillus thuringiensis* (Bt) corn residues and Bt-corn plants affect life-history traits in the earthworm Aporrectodea caliginosa? Applied Soil Ecology 32: 180-187. DOI:10.1016/j.apsoil.2005.07.002.

Verschwele, A. (2011): Is there a Weed Shift in Roundup Ready Maize? (Poster Presentation at the 4th International Workshop PMEM of Genetically Modified Plants, Quedlinburg, Germany 2010): Journal für Kulturpflanzen 63 (7), 203-210.

Verschwele, A., Mülleder, N. (2008): Investigations on weed infestation in the multi-year cultivation of glyphosate-resistant maize. Journal of Plant Diseases and Protection XXI, 57-62.

Vila-Aiub, M.M., Balbi, M.C., Distéfano, A.J., Fernández, L., Hopp, E., Yu, Q., Powles, S.B. (2011): Glyphosate resistance in perennial *Sorghum halepense* (Johnsongrass), endowed by reduced glyphosate translocation and leaf uptake. Pest Management Science, DOI:10.1002/ps.2286.

Virla, E.G., Casuso, M., Frias, E.A. (2010): A preliminary study on the effects of a transgenic corn event on the non-target pest Dalbulus maidis (Hemiptera: Cicadellidae), ScienceDirect.

Viswanath, V., and Strauss, S.H. (2011): Global regulatory burden for field testing of genetically modified trees. Tree Genetics and Genomes. http://www.springerlink.com/content/528618865031865j/

Voet van der, H., Perry J.N., Amzal, B., Paoletti, C. (2011): A statistical assessment of differences and equivalences between genetically modified and reference plant varieties. BMC Biotechnology 2011, 11:15.

Vogler, U., Rott, A.S., Gessler, C., Dorn, S. (2009): Comparison between volatile emissions from transgenic apples and from two representative classically bred apple cultivars, Transgenic Research 19, 77-89.

Volkmar, C., Hussein, M.L.A., Jany, D., Hunold, I., Richter, L., Kreuter, T., Wetzel, T. (2003): Ecological studies on epigeous arthropod populations of transgenic sugar beet at Friemar (Thuringia Germany). Agriculture, Ecosystems and Environment 95: 37-47.

Waltz, E. (2010): Glyphosate resistance threatens Roundup hegemony. Nature Biotechnology 28, 537-538.

Wan, S., Norby, R.J., Ledford, J., Weltzin, J.F. (2007): Responses of soil respiration to elevated CO2; air warming; and changing soil water availability in a model old-field grassland. Global Change Biology 13: 2411-2424. DOI:10.1111/j.1365-2486.2007.01433.x.

Wandeler, H., Bahylova, J., Nentwig, W. (2002): Consumption of two Bt and six non-Bt corn varieties by the woodlouse Porcellio scaber. Basic Appl. Ecol. 3: 357-365.

Wang, H., Ye, Q., Wang, W., Wu, L., Wu, W. (2006b): Cry1Ab protein from Bt transgenic rice does not residue in rhizosphere soil. Environmental Pollution 143: 449-455. DOI:10.1016/j.envpol.2005.12.006.

Wang, S., Rust, D.R., Pinstrup-Andersen, P. (2006a): Tarnishing silver bullets: Bt technology adoption, bounded rationality and the outbreak of secondary pest infestations in China. American Agricultural Economics Association Meeting. Long Beach, Canada.

Warwick, S., Beckie, H.J., Simard, M.J., Légère, A., Nair, H., Séguin-Swartz, G. (2004): Environmental and agronomic consequences of herbicide-resistant (HR) canola in Canada. In: den Nijs, H.C.M., Bartsch, D., Sweet, J. (Eds), Introgression from Genetically Modified Plants into Wild Relatives (pp 323-337), CABI publishing.

Warwick, S.I., Beckie, H.J., Small, E. (1999): Transgenic crops: new weed problems for Canada? Phytoprotection, 80: 71-84.

Warwick, S.I., Légère, A., Simard, M.J., James, T. (2008): Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy Brassica rapa population. Molecular Ecology 17: 1387-1395. DOI:10.1111/j.1365-294X.2007.03567.x.

Warwick, S.I., Simard, M.J., Légère, A., Beckie, H.J., Braun, L., Zhu, B., Mason, P., Séguin-Swartz, G., Stewart, C.N. (2003): Hybridization between transgenic Brassica napus L. and its wild relatives: Brassica rapa L., Raphanus raphanistrum L., Sinapis arvensis L., and Erucastrum gallicum (Willd.) O.E. Schulz. Theoretical and Applied Genetics 107: 528-539.

Watrud, L.S., King, G., Londo, J.P., Colasanti, R., Smith, B.M., Waschmann, R.S., Lee, E.H. (2011): Changes in constructed Brassica communities treated with glyphosate drift. Ecological Applications 21 (2), 525-538.

Weber, C.R., Hanson, W.D. (1961): Natural hybridization with and without ionizing radiation in soybeans. Crop Science 1: 389-392.

Webster, T.M., Sosnoskie, L.M. (2010): Loss of Glyphosate Efficacy: A Changing Weed Spectrum in Georgia Cotton, 58 73-79.

Wei, J.Z., Hale, K., Carta, L., Platzer, E., Wong, C., Fang, S.C., Aroian, R.V. (2003): Bacillus thuringiensis crystal proteins that target nematodes. Proceedings of the National Academy of Sciences of the USA 100: 2760-2765. DOI:10.1073ypnas.0538072100.

Weider, C., Stamp, P., Christov, N., Hüsken, A., Foueillassar, X., Camp, K.H., Munsch, M. (2009): Stability of Cytoplasmic Male Sterility in Maize under Different Environmental Conditions. Crop Science 49, 77-84.

Wellington, E.M.H., Elsas, J.D. van, (eds.) (1992): Genetic interactions among microorganisms in the natural environment. Pergamon Press. Oxford.

Wennström, A. (2004): Risk assessment of Genetically Modified undomesticated plants. In: Den Nijs, H., Bartsch, D., and Sweet, J. (eds.) Introgression from Genetically Modified Plants into wild relatives and its consequences, CABI Wallingford, 297-307.

Whalon, M.E., Mota-Sanchez, D., Hollingworth, R.M., Duynslager, L. (2008): Arthropod pesticide resistance database. Michigan State University, 2004-2008. http://www.pesticideresistance.org (accessed on 21 January, 2009)

Whalon, M.E., Mota-Sanchez, D., Hollingworth, R.M., Duynslager, L. (2011): Arthropod pesticide resistance database. Michigan State University, 2004-2011, http://www.pesticideresistance.org

White, S.M., Rohani, P., Sait, S.M. (2010): Modelling pulsed releases for sterile insect techniques: fitness costs of sterile and transgenic males and the effects on mosquito dynamics. Journal of Applied Ecology. DOI:10.1111/j.1365-2664.2010.01880.x.

Widmer, F. (2007): Assessing effects of transgenic crops on soil microbial communities. Advances in Biochemical Engineering/Biotechnology 107: 207-234.

Wilhelm, R., Sanvido, O., Castanera, P., Schmidt, K., Schiemann, J. (2010): Monitoring the commercial cultivation of Bt maize in Europe – conclusions and recommendations for future monitoring practice, Environmental Biosafety Research.

Wilkinson, M.J., Elliott, L.J., Allainguillaume, J., Shaw, M.W., Norris, N., Welters, R., Alexander, M., Sweet, J., Mason, D.C. (2003): Hybridization between Brassica napus and Brassica rapa on a national scale in the United Kingdom. Science 302: 457-459.

Wilson, R.G., Young, B.G., Matthews, J.L., Weller, S.C., Johnson, W.G., Jordan, D.L., Owen, M.DK., Dixon, P.M., Shaw, D.R. (2011): Benchmark study on glyphosate-resistant cropping systems in the United States. Part 4: Weed management practices and effects on weed populations and soil seedbanks. Pest Management Science. DOI:10.1002/ps.2176.

Woiwod, I.P. and Schuler, T.H. (2007): Genetically modified crops and insect conservation, Rothamsted Research. In: Stewart, A.J.A., New, T.R., Lewis, O.T. (Eds); Insect Conservation Biology: The 22nd Symposium of the Royal Entomological Society. CABI Publishing.

Wold, S.J., Burkness, E.C., Hutchison, W.D., Venette, R.C. (2001): In-field monitoring of beneficial insect populations in transgenic corn expressing a Bacillus thuringiensis toxin. Journal of Entomological Science 36: 177-187.

Wolfenbarger, L.L., Naranjo, S.E., Lundgren, J.G., Bitzer, R.J., Watrud, L.S. (2008): Bt crop effecs on functional guilds of non-target arthropods: a meta-analysis. PLoS ONE 3: 1-11 (e2118).

Wolt, J.D., Keese, P., Raybould, A., Fitzpatrick, J.W., Burachike, M., Gray, A., Heron, D.S., Olin, S.S., Schiemann, J., Sears, M., Wu, F. (2010): Principles of environmental risk assessment for genetically modified plants: Problem formulation. A Report from the ILSI Research Foundation Expert Consultation on Problem Formulation for ERA of GM Crops. Transgenic Research 19(3): 425-436.

Wolt, J.D., Peterson, R.K. (2010): Prospective formulation of environmental risk assessments: Probabilistic screening for Cry1A(b) maize risk to aquatic insects, Ecotoxicology and Environmental Safety.

Wraight, C.L., Zangerl, A.R., Carroll, M.J., Berenbaum, M.R. (2000): Absence of toxicity of Bacillus thuringiensis pollen to black swallowtails under field conditions. Proceedings of the National Academy of Sciences of the USA 98: 11908-11912. DOI:10.1073/pnas.130202097.

Wu, K. (2010): No refuge for insect pests. Nature Biotechnology 28 (12).

Wu, W., Su, Q., Xia, X.Y., Wang, Y., Luan, Y.S., An, L.J. (2008): The Suaeda liaotungensis kitag betaine aldehyde dehydrogenase gene improves salt tolerance of transgenic maize mediated with minimum linear length of DNA fragment. Euphytica 159: 17-25. DOI:10.1007/s10681-007-9451-1.

Wu, Y.Y., Chen, Q.J., Chen, M., Chen, J., Wang, X.C. (2005): Salt-tolerant transgenic perennial ryegrass (Lolium perenne L.) obtained by Agrobacterium tumefaciens-mediated transformation of the vacuolar Na+/H+ antiporter gene. Plant Science 169: 65-73.

Xue, R.G., Zhang, B., Xie, H.F. (2007): Overexpression of a NTR1 in transgenic soybean confers tolerance to water stress. Plant Cell, Tissue and Organ Culture 89: 177-183. DOI:10.1007/s11240-007-9231-6.

Yang, X., Harrison, S.K., Riedel, R.M. (2002): Soybean (Glycine max) response to glyphosate and soybean cyst nematode (Heterodera glycines). Weed Technology 16: 332-339. DOI:10.1614/0890-037X(2002)016[0332:SGMRTG]2.0.CO;2.

Yang, X., Xia, H., Wang, W., Wamg, F., Su, J., Snow, A.A., Lu, B.-R. (2011): Transgenes for insect resistance reduce herbivory and enhance fecundity in advanced generations of crop-weed hybrids of rice. Evolutionary Applications. DOI:10.1111/j.1752-4571.2011.00190.x.

Yanni, S.F., Whalen, J.K., Ma, B.L. (2011): Field-grown Bt and non-Bt corn: yield, chemical composition, and decomposability. Agronomy Journal 103, 486-493.

Yeo, E.T., Kwon, H.B., Lee, J.T., Ryu, J.C., Byo, M.O. (2000): Genetic engineering of drought resistant potato plants by introduction of the trehalose-6-phosphate synthase (TPS1) gene from Saccharomyces cerevisiae. Molecules and Cells 10: 263-268.

Young, B.G. (2006): Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technology 20: 301-307.

Yu, Q., Cairns, A., Powles, S. (2007): Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a Lolium rigidum biotype. Planta 225, 499-513.

Zablotowicz, R.M., Reddy, K.N. (2004): Impact of glyphosate on the Bradyrhizobium japonicum symbiosis with glyphosate-resistant transgenic soybean: a minireview. Journal of Environmental Quality 33: 825-831.

Zablotowicz, R.M., Reddy, K.N. (2007): Nitrogenase activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean. Crop Protection 26: 370-376. DOI:10.1016/j.cropro.-2005.05.013.

Zeller, S.L., Kalinina, O., Brunner, S., Keller, B., Bernhard, S. (2010): Transgene x Environment Interactions in Genetically Modified Wheat. Plus One 5 (7), DOI:10.1371/journal.pone.0011405.

Zemková-Rovenská, G., Zemek, R., Schmidt, J.E.U., Hilbeck, A. (2005): Altered host plant preference of Tetranychus urticae and prey preference of its predator Phytoseilus persimilis (Acari: Tetranychidae, Phytoseiidae) on transgenic Cry3Bb-eggplants. Biological Control 33: 293-300.

Zhao, J.Z., Collins, H.L., Tang, J.D. Cao J., Earle, E.D., Roush, R.T., Herrero, S., Escariche, J., Ferré, J., Shelton, A.M. (2000): Development and characterization of diamondback moth resistance to transgenic broccoli expressing high levels of Cry1C. Applied and Environmental Microbiology 66: 3784-3789.

Zhao, J.Z., Lu, M.G., Fan, X.L., Wi, C., Liang, G.M., Zhu, C.C. (1996): Resistance monitoring of Helicoverpa armigera to Bacillus thuringiensis in North China. Pest Management Science 8: 20-21.

ZKBS (2011): Statement of the Central Commission on Biological Safety (ZKBS) on the latest scientific publications on the risk assessment of the maize line MON810. http://www.bvl.bund.de/EN/06_Genetic_Engineering/ZKBS/01_Allg_Stellungnahmen/05_plants/zkbs_plants_maize_MON810_statement_2011.pdf?__blob=publicationFile&v=4

Zuo, J., Zhang, L., Song, X., Dai, W., Qiang, S. (2011): Innate factors causing differences in gene flow frequency from transgenic rice to different weedy rice biotypes. Pest Management Science. DOI:10.1002/ps.2108.

Zurbrügg, C., Hönemann, L., Meissle, M., Romeis, J., Nentwig, W. (2010): Decomposition dynamics and structural plant components of genetically modified Bt maize. Transgenic Research 19, 257-267.

Zurbrügg, C., Nentwig, W. (2009): Ingestion and excretion of two transgenic Bt corn varieties by slugs. Transgenic Research 18, 215-225.

Zuver, K.A., Bernards, M.L., Kells, J.L., Sprague, C.L., Medlin, C.R., Loux, M.M. (2006): Evaluation of postemergence weed control strategies in herbicide-resistant isolines of corn (Zea mays). Weed Technology, 20: 172-178.

Zwahlen, C., Andow, D.A. (2005): Field evidence for the exposure of ground beetles to Cry1Ab from transgenic corn. Environmental Biosafety Research 4: 113-117. DOI:10.1051/ebr:2005014.

Zwahlen, C., Hilbeck, A., Gugerli, P., Nentwig, W. (2003a): Degradation of the Cry1Ab protein within transgenic Bacillus thuringiensis corn tissue in the field. Molecular Ecology 12: 765-775.

Zwahlen, C., Hilbeck, A., Howland, R., Nentwig, W. (2003b): Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Molecular Ecology 12: 1077-1086.

Zwahlen, C., Hilbeck, A., Nentwig, W. (2007): Field composition of transgenic Bt maize residue and the impact on non-target soil invertebrates. Plant and Soil 300: 245-257. DOI:10.1007/s11104-007-9410-6.

Annex Co-Existence Report SIGMEA

Sustainable Introduction of GM Crops Into European Agriculture: a Summary Report of the FP6 SIGMEA Research Project

Antoine Messéan, Geoff Squire, Joe Perry, Frédérique Angevin, Manuel Gomez, Peter Townend, Christophe Sausse, Broder Breckling, Stephen Langrell, Saso Dzeroski and Jeremy Sweet

Keywords: GMOs, gene flow, coexistence, modelling, decision-making, ecological impacts.

Introduction

Genetically-modified (GM) plants are now widely cultivated in North and South America, and to a lesser extent in Asia. In Europe, only a few tens of thousands of hectares of Bt maize are being grown, mostly in Spain. The regulatory provisions of Directive 2001/18 reinforced the prior evaluation of GM crops, set up rules concerning traceability and labeling and imposed post-market monitoring. Furthermore, the European Commission laid down guidelines defining the context for the coexistence of different types of agriculture: “to ensure that each farmer can make a practical choice between conventional, organic and genetically modified (GM) crop production”.

In order to determine what is needed for the sustainable introduction of GM crops in Europe, the cross-disciplinary SIGMEA Research Project was set up to create a science-based framework to inform decision-makers. SIGMEA has (i) collated and analysed European data on gene flow and the environmental impacts of the major crop species which are likely to be transgenic in the future (maize, rapeseed, sugar beet, rice, and wheat), (ii) designed predictive models of gene flow at the landscape level, (iii) analysed the technical feasibility and economic impacts of coexistence in the principal farming regions of Europe, (iv) developed novel GMO detection methods, (v) addressed legal issues related to coexistence, and (vi) proposed public and farm scale decision-making tools, as well as guidelines regarding management and governance.

SIGMEA brought together the principal teams and thereby the principal programmes, studying gene flow in a large number of countries across Europe, representing a wide range of agricultural systems including organic cropping. Seven regional case studies were carried out for designing and assessing scenarios for coexistence.

The largest collection in Europe of data on gene flow and persistence has been organized.

SIGMEA collated and synthesized experimental data on gene flow and filled gaps in knowledge by designing and conducting further evaluations, particularly at the landscape-scale or over several years of cropping sequence. Maize and oilseed rape were the major crops targeted for this study – other crops under consideration were sugar beet, rice and wheat. Available information from past and current field studies on cross pollination, volunteers, ferals and wild relatives were gathered from 22 SIGMEA partners through a rigorous procedure which ensured quality control through electronic submission of data sets using a standard template or “data-entry” format, thorough checking and retrieval of any missing information, internal review of each data set, and a formal mechanism for completing and “signing off” data sets. The data were made available to other users within SIGMEA through a secure web server.

The synergies within the project led new research studies, using harmonised protocols, on moderate- to long-distance gene flow, plant demography and characterising volunteer, feral and wild populations. These new studies led to further SIGMEA datasets in 2006/07. By the end of the SIGMEA project, the database had over 100 data sets (Fig. A), constituting more than 150 "experiment-years". Around two thirds of the data are for oilseed rape or close relatives. Information for beet and maize comprised just less than one sixth each. A few data sets were submitted on wheat and rice. Data on crops and volunteers constitute around 35% each, wild relatives 16% and ferals 6%. However, data on ecological impacts – as distinct from gene flow by seed and pollen – form a small part of the total (as expected in the original proposal); formal submissions in this topic involve Bt maize in Spain and herbicide tolerant oilseed rape.

Due to the very high replication achieved by combining data from different sites, the crop-specific conclusions in SIGMEA on cross pollination and seed persistence in maize and oilseed rape are mostly of very high statistical significance and make it possible to draw general conclusions about given topics. Most of the data sets provide information on scale, climate, geography, biology, as well as spatial and temporal factors associated with pollen flow, cross pollination and seed dynamics, in more detail than appears in refereed publications. The datasets have been extensively used to provide added value through meta-analysis, data mining and the development and verification of gene flow models designed within SIGMEA. Since much of the research is still not in the public domain, the datasets are presently accessible only within SIGMEA to partners who submitted data, and to others with designated access.

In summary, the database, together with information already published, provided a sound basis to enable SIGMEA to reach a set of conclusions with respect to each of maize, oilseed rape and beet as summarised below. Additionally, the database allowed an assessment of three questions about transferability of information: the consistency of measurements at different spatial (or temporal) scales; the differences between agricultural regions in Europe with different climates and soils; and. the behaviour of different crop species.

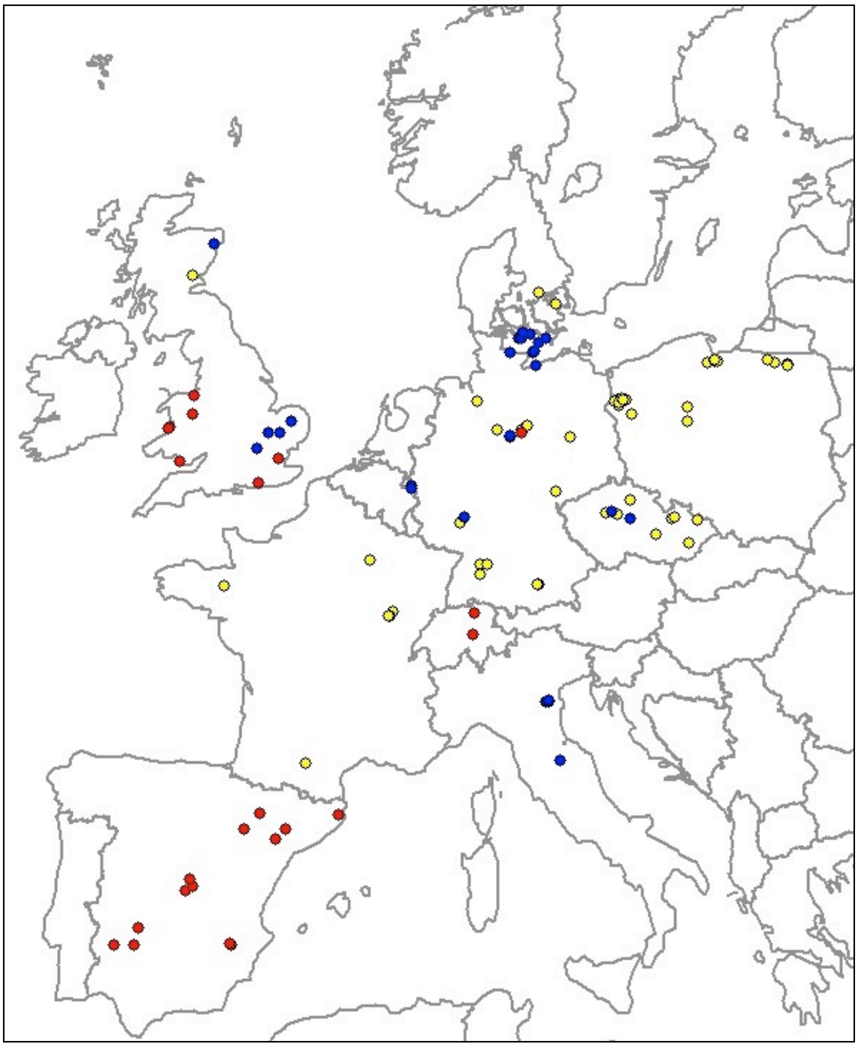


Figure A. Locations of main experimental studies available in the SIGMEA database on maize (red), oilseed rape (yellow) and beet (blue). Some locations involved several experiments.

Enhanced understanding of gene flow informs practical strategies for coexistence in maize, oilseed rape and sugar beet.

Similar biological mechanisms govern the life histories of all crop plants. They produce structures that survive over time and disperse over space and by these means have the potential to transfer genes from one crop to another. Seed-borne genetic impurities can arise by several routes: from plants already present in the field as volunteers (weeds of the same species as the crop) and wild relatives; by seed brought to the field in the sown seed or on farm machinery; and by seed dispersed from feral plants or wild relatives growing around the the field. Volunteers or wild relatives growing in the same field can contribute their own seed to the harvest. Pollen-borne genetic impurities can arrive from another crop and from volunteers, ferals and wild relatives. The seed-borne genetic impurities can arise at any time of the year and from crops grown in the past, while pollen transmission occurs during the relatively short period that both receptor and donor plants are in flower.

Maize

Experiments relevant to coexistence of maize in Europe were almost exclusively on cross pollination between crops, since admixture through seeds and pollen from volunteers was thought to be low and relevant only in warmer regions. Maize has no wild relatives and few feral plants are found in Europe. However maize landraces are maintained in some regions, so special consideration was given to the cumulative outcrossing which may occur between modern varieties and landraces.

Cross pollination has been examined in great detail in several European countries, either using GM crops as a donor or using markers such as yellow and white grain colour. The database allows comparisons across scales, from small plots to full sized commercial fields, and in several contrasting climates in Germany, Spain, Switzerland and the UK. The studies are consistent and indicate a steep decline in cross pollination over three orders of magnitude (a 1000-fold) with distances to 100 m from the source of pollen, and an effect on percentage pollination of wind direction and related meterological factors. Cross pollination declined with distance in a similar manner in both experimental plots and full-sized fields. At 100 m from the donor, cross pollination was below 0.1% in most circumstances. Where donor and receptor fields were well dispersed in a landscape, and at a generally low overall density, the average cross pollination was typically 0.01% between 100 m and several kilometers. Where donor and receptor fields were grown close together in similar proportions (as in Spain, a region where commercial crops are grown without coexistence measures), cross pollination rates above 0.9% were sometimes found in situations where non-GM fields were completely surrounded by GM fields and both types flowered at the same time.

In summary, the potential to introduce adventitious presence of GM material in non-GM maize production is:

- moderate for cross pollination between fields, and can be managed through separation, discards or buffers where crops are in close proximity;
- low through volunteers, and then mainly in southern Europe;
- low for introgression to landraces from modern crop varieties;
- zero through wild relatives as none exist in Europe.

Over most of Europe, therefore, the biology, environment and agronomy of maize have been well characterised, so that coexistence (meeting the official threshold) for hybrid varieties should be achievable by using high purity seed and management of cross pollination by using varieties that flower at different times, spatially separating fields, or using buffers or discards where fields are in close proximity. However, a zero level of adventious presence cannot be achieved or measured in practice. Volunteer maize still needs to be investigated thoroughly in climates where it occurs.

Oilseed rape

Genetic impurities in oilseed rape can arise from a wider range of sources than in maize. Pollen is dispersed by wind, hive bees, bumble bees and a variety of other insects. Transfer by seed following seed drop at harvest can be very high, as large seedbanks can form which survive for several years producing volunteers in subsequent crops. Also seed is transported on farm machinery, from which the small seeds are difficult to remove under normal agricultural conditions. Feral plants are widespread along waysides and margins, while wild relatives, notably *Brassica rapa* (the wild turnip), occur locally and cross pollination with crops, volunteers and ferals.

In total, results from over 50 field-experiments on oilseed rape from the Czech Republic, Denmark, France, Germany, Poland and the UK were re-examined in SIGMEA. Results on cross-pollination differed according to experimental designs, regions, cultivars and climates, but a dispersal function with a ‘fat’ tail (power-law) appears to be the most appropriate currently available to predict pollen movement at any scale. Over distances of tens of metres, cross pollination showed a similar decline to that in maize, and was typically less than 0.1% at 100 m from the edge of the donor; but crossing between commercial fields was sometimes as high as 0.1% even at distances between 100 m and 1000 m. The contribution of volunteers to admixture of harvested seed may range from <0.01% to more than 10% for the same crop variety in different management, soil and climatic conditions A range of agronomic practices can be deployed to limit transfer through seed banks such as delaying soil cultivation after harvest to allow germination and destruction of seedlings, increasing the interval between crops and stale-seedbed techniques. Comparison of feral oilseed rape in more than 20 growing seasons across 5 study areas enabled the definitive statement that, though widespread and sometimes persisting in the same place over several years, ferals are a negligible fraction of the total flowering oilseed rape in a region and contribute little to admixture in crops. The abundance of wild relatives differs between regions, and while their progeny may be fertile and as ecologically fit as the parents, they do not constitute a major route for transmission of traits between to crops.

In summary, the potential to introduce adventitious presence of GM material in non-GM oilseed rape production is:

- moderate for cross pollination between fields, which can be managed through spatial separation and use of buffers or discards where crops are in close proximity;

- high through seedbanks resulting in volunteer populations that admix with and pollinate non-GM crops – volunteers are ubiquitous, mobile and commonly in high abundance and are of maximum importance to coexistence over time;

- moderate through wild relatives in those localised areas of Europe where they occur in high abundance in the fields (e.g., *B. rapa* in Denmark)

- low through ferals (with some local exceptions) because of their low overall density compared to crops and volunteers in the landscape.

Problems of coexistence during the first few years of commercialisation can be reduced by management of cross pollination through separation and seed purity. However uncertainties remain over whether the cumulative movement and amplification of volunteers can be managed so as to achieve coexistence in of GM and non-GM oilseed rape in the longer term.

Beet

Crop varieties, in-field volunteers, ferals and wild types of beet are all sexually compatible variants of the species, *Beta vulgaris,* and together comprise the Beta complex. Crop beet plants are biennial, producing root bulk in the first season (after which they are usually harvested) and flowers in the second. By contrast most wild and weed beet forms are annual, producing flowers in the year they germinate. Flowers produce small wind-borne pollen that can disperse over large distances. The main source of genetic impurity in commercial crops arises from seed produced in localised areas of Italy and France in fields consisting of male fertile pollinators and male sterile seed mother plants. The male sterile mother plants can also receive pollen from volunteers, ferals and wild sea beet in the surrounding countryside and from other seed production fields in the area. The wild and weedy forms introduce annual genes into the seed crop, which give rise to annual plants that flower in the first year of the crop but produce little or no root and sugar yield. If allowed to set seed, these annual weedy beets give rise to seedbanks lasting many years, from which annual volunteers (bolters) will flower.

Annual traits, whether GM or otherwise, have the potential to spread in commercial production areas, but as indicated above, annual plants rarely give rise to tubers and so contribute little to adventitious presence in sugar beet. Their main importance is as weed. If herbicide tolerant (HT) beets are grown, HT weed beets will arise and pollinate non-GM weed beets and in this way introduce HT genes into non-GM fields. Since this does not translate in adventitious presence of GM in the final crop (roots), and therefore is not a coexistence issue *sensu stricto*, it could create weed management problems. For example, if the HT trait conferred tolerance to glyphosate, this same herbicide would become less effective for weed beet control in the non-GM beet crops.

SIGMEA drew together current and recent research on the Beta complex. Compared to maize and oilseed rape, there is little data on the form of the decline in cross pollination with distance, though in the studies examined pollen was found to move over at least several hundred metres. The work on beet in SIGMEA concentrated on weed and wild beet. Unlike in the other two species examined, the wild form, sea beet, is an important genetic resource within the *Beta* complex, and is used as a source of genetic traits by plant breeders. Genetic assessment of plants growing along both the Baltic and Adriatic coasts, confirmed populations remain highly diverse and distinct from crop varieties. Nevertheless, areas were identified where the crop, volunteer, feral and wild beets exist in proximity and exchange genetic material through movement of seed and pollen. It is considered essential to preserve the diversity of sea beet for any long term, plant breeding strategy, and for conservation and study in its own right.

In summary, the potential for adventitious presence of GM material in non-GM sugar beet production is:

- low through cross pollination between sugar beet crops since the harvest is vegetative,
- low through volunteer (weed beet) populations which arise from impurities in sown seed, since best management should minimise any harvest contamination with roots of these weed beets;
- low though cross pollination from feral plants and wild beet for the reasons given for volunteers.

The main source of adventitious presence is therefore through the seed sown to grow crops of sugar beet. Coexistence should still be achievable by best management of seed production crops, and by strategic siting and separation of seed production fields. Specifically, GM seed production crops need to be sufficiently separated from non-GM crops and from wild and weedy beet (which in time would contain GM individuals) both to keep the non-GM seed pure and to reduce the spread of transgenes into wild, weedy and feral populations. Separate areas or regions for GM and non-GM seed production may be required.

Wheat and rice

The knowledge-base for wheat and rice in Europe is much less than for the other crops, but tentative conclusions are that the potential for introducing impurities should be:

- low through cross pollination between crops,
- probably low in rice (to moderate in wheat) through volunteers, but their contribution needs to be clarified under European conditions;
- low in wheat through wild relatives, and low to moderate in rice through the red rice weed, in those areas where it occurs, provided agricultural practices to control this weed are applied.

Further research is needed on cross pollination and the life cycle of these species and their wild relatives in Europe.

General

In summary, the general conclusion drawn from gene flow studies of maize, oilseed rape and beet is that adventitious presence due to cross pollination alone can generally be managed through separation distance and related measures to comply with the official EU regulation. However it should be recognised that zero level of adventious presence cannot be achieved or measured in practice.

Oilseed rape (OSR) was identified by SIGMEA as having major problems in the management of coexistence. The problems arise principally because OSR seeds survive for several years in soil and give rise to volunteers that are competitive and difficult to eliminate. Thus gene movement and persistence in seeds and volunteers is difficult to manage agronomically. Coexistence issues arising from maize volunteers are manageable using good agricultural practice. The problems associated with weed beet are mostly related to seed production which therefore needs careful management on a regional scale.

There remains uncertainty on the relevance to coexistence of transgenes that might confer differential fitness, for example by being associated with reduced pollen production or resistant to common herbicides. Further measurements at previous GM release sites are needed to assess the persistence and genetic structure of relevant populations (e.g. volunteers, wild relatives). State of the art modelling tools (individual based, spatially explicit, incorporating introgression of multiple events) have been developed to simulate the population dynamics around complex transgenic events, and could be adapted as aids to monitoring following commercialisation.

A synthesis of available data on environmental impacts of Bt maize and HT oilseed rape within European cropping systems

SIGMEA reviewed the (a) impacts of gene flow and introgression on within-and-between-species plant diversity and (b) the wider ecological implications of growing Bt maize and HT oilseed rape. It linked several important ‘impact’ studies, notably those in Spain on Bt maize[10] and in the UK on HT beet, maize and oilseed rape[11], and was closely associated with the EU ECOGEN project on Bt maize[12].

The approaches to studying environmental impacts in SIGMEA were based on the key elements described in the US Environmental Protection Agency Guidelines of 1998 and the European Food Safety Authority Guidance Document of 2006. The “exposure” and the “effect” were considered for a range of ecological indicators of the in-field soil and food web, including soil biophysical status, soil micro- and meso-fauna, plant species, functional groups and assemblages (as affected by introgression and field management, e.g. herbicide), plant-feeding invertebrates and other invertebrate functional or trophic groups. There was little evidence available to SIGMEA (and little evidence generally) of wider effects on, for example, biogeochemical cycles and the quality of water or air. The conclusions reached by SIGMEA for the main crops studied are as follows:

---

[10] Monitoring programme of Bt maize in Spain: Farinos et al., 2008. Diversity and seasonal phenology of aboveground arthropods in conventional and transgenic maize crops in Central Spain. Biological Control, Volume 44, Issue 3, March 2008, Pages 362-371.

[11] Farm Scale Evaluation: Firbank et al., 2003. An introduction to the Farm Scale evaluations of genetically modified herbicide-tolerant crops. Journal of Applied Ecology 40, 2-16.

[12] FP5 project ECOGEN (www.ecogen.dk/reports) – PH Krogh & B. Griffiths, 2007. ECOGEN: soil ecological and economic evaluation of Genetically Modified Crops. Pedobiologia 51 (2007) 171-173.

- Maize (Bt varieties, targeted at corn borers). There appears to be no reason on grounds of biosafety not to increase the scale of growing. The most consistent finding is that Bt maize in field trials and crop production in Europe to date had no systematic or reproducible effects on any of the invertebrates or soil organisms studied over a time period of several years. In contrast, over similar time periods, other agronomic factors did have large and measurable effects on the same organisms. Appropriate monitoring should be in place, especially for resistance development in corn borers, and potential effects on certain sensitive non-target biotic groups should be considered in greater depth.

- Oilseed rape (HT varieties, tolerant to glufosinate ammonium or glyphosate). The ranking of HT oilseed rape against the comparator, usually the conventional crop and agronomy, varied with the local context. Negative effects occurred where a) the herbicides used in HT cropping caused a systematic depletion of the weed flora and dependent invertebrates resulting in reductions in biodiversity within fields, and b) the presence of HT volunteers limited future options for use of herbicides and the growing of certain crops such as beans in which volunteers are difficult to control. Positive effects may occur due to the herbicides used with HT cropping being less toxic to non-weed organisms than most other herbicides and crop protection chemicals. Nevertheless, the ecological effects of HT crops compared to non-HT in the same production system are generally smaller than those due to differences between crop species, season of sowing or agronomic practices.

- Beet. The various types of beet – crop, weed, feral, wild – are in genetic contact through seed and pollen. Wild beet needs proactive conservation, since it is a biologically interesting plant form of restricted habitat, a source of genes for future beet breeding and a source of annual impurities in crop beets. HT beet cultivation could also deplete biodiversity within fields for the same reasons as discussed for HT oilseed rape.

In summary, statistically significant effects of GMHT cropping on ecological processes or organisms have been obtained in the field, but most effects are smaller than or at most comparable to those due to general agronomic operations. There is an increasing consensus that future assessment of GM crops considers both negative and positive impacts of GM cropping in a more holistic way than previously. Most important, standards and criteria for environmentally resilient cropping systems are needed against which GM cropping and its non-GM comparator can be assessed. Setting such environmental standards is now an absolute priority.

A landscape generator simulating agricultural landscapes has been designed and is available online.

SIGMEA designed LandSFACTS, a user-friendly windows-based software to simulate crop allocation to fields by integrating typical crop rotations and crop spatio-temporal arrangements within agricultural landscapes. LandSFACTS reproduces the farmers' decision-making process for crop succession and location (rotational and spatial rules). Rules on rotational (equivalent to temporal), spatial and spatio-temporal patterns of crops in agricultural landscapes were determined by analysing existing data from SIGMEA case study areas, by analysing the questionnaires to farmers on the decision process for growing specific crops on specific fields and their links to agronomical and economical rules, and by analysing results of discussions with farmers' advisers. Specific modelling algorithms for simulating crop allocation to fields in a realistic and reliable way were created.

The general structure of LandSFACTS, its interfaces with Geographical Information Systems (GIS) and the generic gene flow platform LandFlow-Gene as well as its user interface were set up through a close liaison with modellers and case studies to ensure its usefulness and quality.

The final version of LandSFACTS was released in June 2007 as open-source software under the GNU Public Licence and is publicly available at http://www.rothamsted.bbsrc.ac.uk/pie/LandSFACTS/.

In summary, LandSFACTS generates an agronomic arena that can act as the input for other research tools, especially models, and for informing various issues related to to spatial agricultural processes. Indeed, agricultural models often need to operate at large spatial scales, such as landscapes or regions over many years. LandSFACTS facilitates the setting up of realistic scenarios at such scales.

An operational, practical and dynamic generic gene flow modelling platform LandFlow-Gene is available for research purposes

A generic gene flow platform has been designed and validated for research purposes. LandFlow-Gene allows users to evaluate the effects of landscapes, climate, cropping systems, agricultural practices on gene flow and adventitious presence of GM material in non-GM production. LandFlow-Gene is operational for maize and oilseed rape through the use of two previously existing models: MAPOD® (Maize) and GeneSys (Oilseed Rape). These models have been further validated within SIGMEA and have benefited from the largest available data sets collated in Europe in an improved capacity to assess and predict levels of gene flow between crops.

Interfaces with GIS-datasets and the Landscape Generator LandSFACTS are available (figure B).

LandFlow-Gene thus provides tools to run spatial and temporal simulations of pollen and seed dispersal for rapeseed and maize crops. Given an agricultural landscape, a climate, cropping systems and crop management practices, LandFlow-Gene predicts the adventitious presence of GM in non-GM fields under various scenarios of GM adoption. Figure C presents an output of LandFlow-Gene for maize.

LandFlow-Gene was used to analyze the regional case studies of SIGMEA and to support the cost analysis.

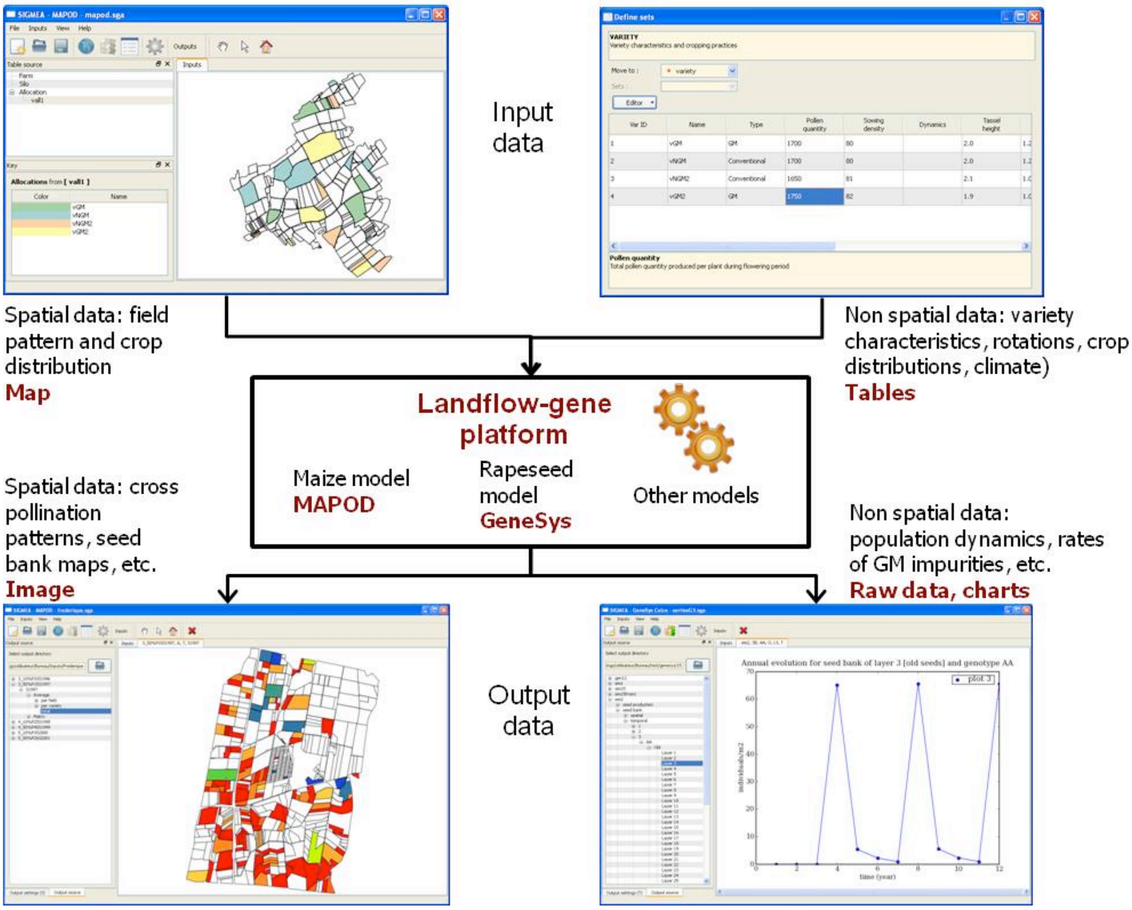


Figure B. General Structure of LandFlow-Gene. LandFlow-Gene uses spatial information on landscape patterns and crop allocation as well as information on crop management, climate and varieties to estimate the proportion of GM material in non-GM crop production. Outputs can be obtained at the field level (average mean of adventitious presence) or for small units (intra-field adventitious presence).

The following software was developed by SIGMEA:

- LandFlow-Gene: complete generic platform software for rapeseed and maize. It can be simply adaptable to other crops (connexion with an .exe file);
- LandFlow-Gene-GeneSys: generic platform for rapeseed;
- LandFlow-Gene-MAPOD: generic platform for maize;
- LandFlow-Gene-Viewer: viewer for LandFlow-Gene outputs;
- Shpconv: converter of shapefile (file coming from GIS) into matricial or vectorial format.

The three first software products include MAPOD® and/or GeneSys© for rapeseed. Access to MAPOD® and GeneSys for research applications is governed by a license agreement under the European agency for programme protection (http://app.legalis.net/) granted to INRA in 2003 and renewed in 2005 (GeneSys) and in 2006 (MAPOD®). This helps to protect INRA in the case of liability issues. Members of the SIGMEA consortium have access to these models if they sign a licence agreement. To date, the use is restricted to research purposes.

The two last programmes were specifically produced by SIGMEA partners. Members of the consortium have free access but distribution outside of the SIGMEA consortium requires agreement from INRA to ensure traceability of uses.

In summary, SIGMEA has developed a generic platform to model gene flow at the scale of agricultural landscapes – LandFlow-Gene. For any agricultural plot described using a geographical information system, this platform can test different scenarios of GM introduction, take account of the effects of practices and the climate, and deliver a diagnosis as to the gene flow. The current version is now operational for maize and rapeseed, and could easily be extended to include other species. In addition, the platform could be adapted to take account of other biological flows, such as spore dispersal. SIGMEA thus makes it possible to answer questions such as “what will happen, in terms of gene dispersal, if a particular GM organism is introduced into a particular European region?” and “how can crops be organised so as to maintain the fortuitous presence of GMOs in conventional crops within the legal thresholds?”

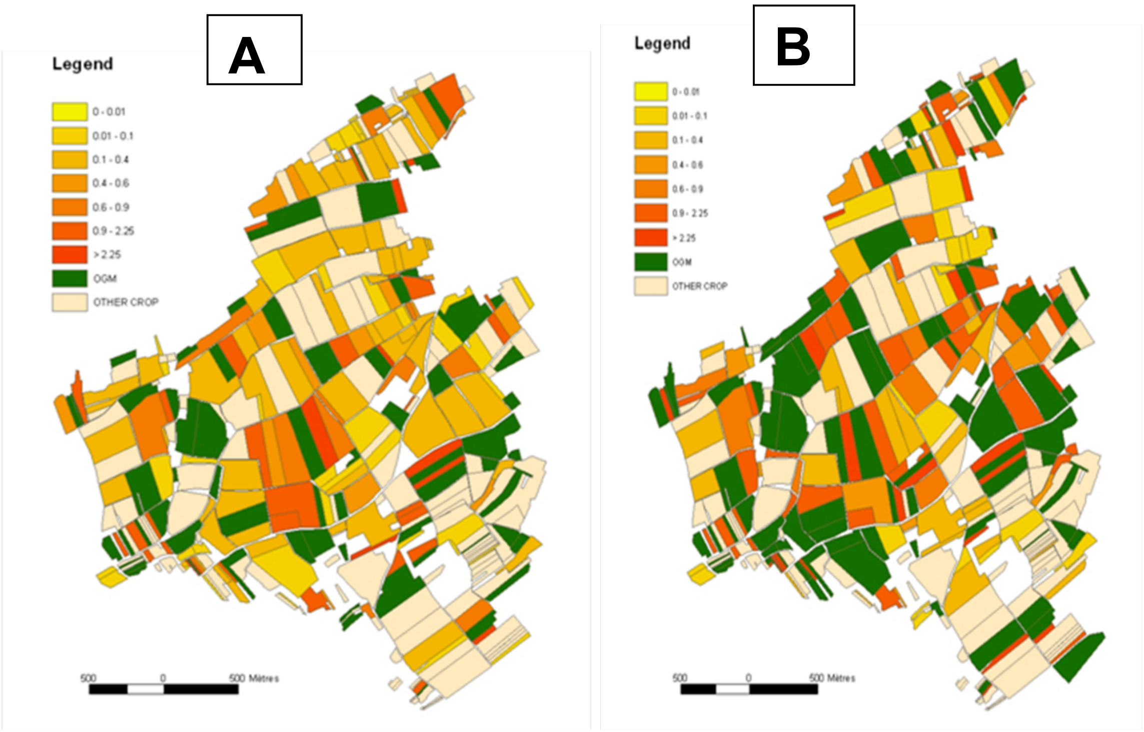


Figure C: Outputs of LandFlow-Gene. This landscape is simulating conditions in Alsace (France) where 70% of the arable land is maize, 10% of maize fields are GM maize. A and B are two different allocations of GM maize: in B the GM fields are less scattered and the overall GM adventitious presence in non-GM fields is lower (0.18%) than in allocation A (0.23%).

The feasibility of coexistence and its costs have been analyzed in various European agricultural situations and scenarios for managing coexistence are proposed.

European Agriculture is diverse and landscapes, climate, cropping systems and crop management practices differ across Europe. Managing coexistence in practice has been studied at the regional level by assessing the impact of growing GM crops on gene flow under various scenarios. Regional case studies were conducted at three embedded scales: whole regions, small agricultural regions corresponding to homogeneous farming systems, and small landscapes of a few km² according to the requirements of the simulation tools.
The approach implemented four steps:

- First, the case studies were described according to all the main variables influencing coexistence.
- Secondly, the impact of structural variables (mainly landscapes and cropping systems) was assessed without any coexistence management measures (using the LandFlow-Gene platform).
- Third, identification and management of critical points were discussed according to the opinions of the main stakeholders and considering their views on constraints and leeways. For this purpose, new data was collated from: 1) surveys carried out with individual farmers, 2) working groups of farmers, collecting firms and advisers, and, 3) the use of a gene flow platform to test the efficiency of certain strategies.
- Finally, the fourth step set up scenarios based on role-playing games allowing stakeholders to discuss realistic management situations. Simulations were used during the games to predict the consequences of different management strategies.

Seven case studies were chosen, but the whole methodology was implemented only for two of them (Table 1). The work carried out in Aragon, Aquitaine and Fife aimed at comparing the effect of structural variables on gene flow and the management of critical points between case studies. Simulations were carried out in Switzerland and Schleswig Holstein to illustrate specific problems or phenomena such as the management of boundaries (Switzerland/France) or dilution effects (Schleswig Holstein). Although Beauce and Alsace were the main studies, generic conclusions were drawn for other regions as well.

Table 1: Regional case studies methodology

Case study	Crop	Description of regional contexts and crop management practices	Assessment of coexistence under current practices	Management of critical points (effect of additional measures)	Elaboration of scenarios
Alsace (France)	maize	🟧	🟧	🟧	🟧
French-Swiss border	maize		🟧		
Aragon (Spain)	maize	🟧	🟧		
Aquitaine (France)	maize	🟧	🟧		
Beauce (France)	OSR	🟧	🟧	🟧	🟧
Fife (Scotland)	OSR	🟧	🟧		
Schleswig Holstein (Germany)	OSR		🟧		

The work carried out suggested a framework to identify and organize the main factors that could determine the implementation of coexistence in specific contexts. These factors fall into three categories:

1. **Structural variables** describing the characteristics of the agroecosystem (cropping systems, landscapes, meteorology, crop management) having an influence on gene flow.

2. **Organizational variables** concerning farmers and grain collecting firms, explaining how they adapt their management according to certain constraints and rooms for manoeuvre. We identified two types of adaptation. First, each actor mobilizes its own resources to various degrees, from technical choices in the short term, to more strategic in the long term: a farmer, for example, may adapt agricultural practices, change his rotations, or decide new investments, while a collecting firm may amend the planning of the grain collection or decide to invest in new storage capacity. Secondly, coordination is crucial, whether between farmers, collecting firms or between farmers and collecting firms. Here arises the question of practical feasibility of collecting and sharing information in a region.

3. Characteristics of the **introduction of GMOs**. Coexistence implementation also depends on market conditions (relative prices of GM and non-GM products on the marketplace), on considered thresholds (which can differ from what is required by regulation, e.g., specific market requirements) and on traits (some traits – e.g., Bt traits which require refugia areas – may facilitate or constrain certain types of coexistence measures).

For given characteristics of GM introduction (crop density, marketshare of GM, threshold), we have highlighted the variability of structural and organizational factors, between regions and within each of them. Maize case studies, for example, have shown that the comparative sensitivity to gene flow was higher inside one region than between two remote European regions (e.g., Alsace and Aragon). In fact, landscape patterns (sizes and shapes of fields) may differ more within one region than between regions and this greatly affects coexistence features.

Based on the simulation results obtained in regional case studies, we have identified four major types of situations, the so-called pre-scenarios[13], that local stakeholders may have to deal with

- segregation at the silo level is feasible without any specific measures at the field level;
- curative measures at harvest (selection of non-GM fields or parts of fields) allows meeting market requirements in terms of targeted thresholds;
- preventive measures at the crop level (e.g., sowing dates) or at the system level (crop rotation, spatial arrangement of crops;
- coexistence is not possible because whatever the agronomic measures undertaken at the crop or system level, the targeted threshold cannot be met or requires non realistic measures.

For a given threshold and a given rate of introduction of GMOs in the landscape, limits between the pre-scenarios are defined by the sensitivity of the landscape to gene flow, as well as the capacity of actors to put them to work. Oilseed rape (OSR) is a particular problem because of the dynamics of volunteers in the cropping system. If farmers wished to return to conventional varieties after GM cultivation, the fields should be managed differently from those which have never been grown with GM OSR. For these latter fields, a thorough control of volunteers will be required in order to meet thresholds. Even if GM and non-GM OSR fields are spatially segregated (i.e., if non-GM varieties are never grown in fields previously cultivated with GM varieties), proper management is required to reduce both spatial and temporal gene flow due to volunteers.

Role-playing games carried out in Alsace and Beauce made it feasible to test the relevance of pre-scenarios under realistic management situations. They demonstrated how players (farmers, collectors) would combine different management strategies in a more or less coordinated way, and how these strategies may evolve over time. It thus appears that risk assessment determines actions, such as the selection of “complying” or “non-complying” quality harvests by the collecting firms according to their presumed GMO content and the targeted threshold firms are considering.

---

[13] The word “pre-scenario” is used because the pre-scenarios only cover a component of the overall picture and should then be integrated into overall management scenarios taking into consideration other factors than those affecting farm coexistence (see below).

Risk assessment and management are not static and evolve according to feedback from experience. We observed that the effectiveness of measures undertaken at the field level was ensured only if the rules (i.e., agreement on the way to assess risks and on the measures to be implemented) were shared between the collecting firms and farmers. In addition, the role-playing games demonstrated that collating and sharing information at the territory level is essential to facilitate coexistence. This raises practical implementation problems that are not currently solved.

Three main processes determine how pre-scenarios may be embedded into global management scenarios:

- the system and rules for collating and sharing information at the territory level,
- the framework and procedures describing coordination between actors,
- and learning processes (both individual and collective).

Based on these findings, contrasting global scenarios may be defined by considering different regulation approaches:

- A “bottom-up” approach, which freely allows the private actors (collector, farmers) to choose the best way to achieve the objectives of coexistence and to meet regulatory or market-based threshold requirements;
- A “top-down” approach, based on the strong intervention of public authorities with the implementation of compulsory uniform measures (e.g., isolation distances).
- and a “third way” approach, which provides a focused response of authorities to lift some constraints on information and coordination between private actors, and allow some flexibility in the measures .

Each approach has advantages and disadvantages: the “bottom up” approach allows more flexible measures than the “top down” one, leading to subsequent lower costs. Moreover, it may help in dealing with management problems out of the scope of the GM regulations, such as specific requirements for “Identity Preserved” (IP) market. However, it may not prevent distrust from the general public and does not solve all the liability issues. The “third way” takes advantage of both local knowledge from individual stakeholders and the ability of public authorities to collect and share information at a large scale, in order to cope with practical problems raised by the implementation of coexistence measures.

Costs of coexistence highly depend on the framework for implementing coexistence measures and uniform measures are not optimal

The economic perspective of coexistence of GM and non-GM crops with specific applicability to oilseed rape (OSR) and maize in different regions of the EU was investigated by SIGMEA. Three levels of coexistence costs were considered:

- Costs of compliance to the coexistence measures developed to prevent adventitious presence of GM material as a result of cross-pollination;
- Monitoring costs (testing for adventitious presence in non-GM crops);
- Costs due to failure of the system (losses due to contamination of conventional crops).

Coexistence costs had already been investigated in former coexistence studies[14]. In addition to standard coexistence measures such as isolation distances, we also considered flexible coexistence measures which allow GM and non-GM crops to be grown in adjacent fields as long as farmers coordinate their activities by:

- implementing a non-GM buffer zone (BZ) within GM fields, large enough to prevent cross-pollination to reach the official thresholds in neighboring fields cultivated with the same crop;
- discarding a non-GM strip (discard zone – DZ) within non-GM fields (again large enough to ensure the remaining parts of non-GM fields comply with thresholds). The crop from the discard strip could be delivered as a GM product by either party involved; the non-GM farmer gets a compensation for the income forgone, either from the GM farmer or from an insurance.
-

Various sizes of buffer and discard zones have been considered (from 10 to 100m[15]). These scenarios require a good coordination between farmers and they were compared to compulsory isolation distances between fields (various distances have also been considered for this measure).

We assumed that GM farmers could benefit from GM technology by saving costs (e.g., herbicides or insecticides) or by higher yields (Bt traits). Different percentages of such benefits were considered. Non-GM farmers could receive a premium in an Identity-Preserved (IP) market and they might want to undertake additional measures to meet such IP requirements, as long as the price premium covers these costs.

The coexistence costs were addressed in the same regional case studies as those considered for assessing the technical feasibility of coexistence:

- coexistence costs for oilseed rape were examined in the Beauce region (France) and in the Fife region (Scotland);
- coexistence costs for maize were discussed in the Aragon region (Spain) and in Alsace (France);
- the potential costs of transboundary coexistence between France and Switzerland were analysed.

For calculating the coexistence costs, spatial simulation models taking into account the economic incentives for coexistence were used. Using a Geographical Information System (GIS) dataset and Arcview® software, a set of simulations of realistic coexistence scenarios were carried out in order to assess the costs of coexistence in the different regions. We assumed that each GM and non-GM field was managed independently but that farmers agreed that buffer zones or discard zones were cultivated with non-GM varieties of the same crop species. It was also assumed that other sources of adventitious presence were controlled (e.g., no GM presence in non-GM seeds, or in volunteers in non GM crops)[16].

---

[14] See for example Bock et al, 2002 and Messéan et al., 2006.

[15] Large sizes of BZ or DZ have been considered as they would drastically reduce cross pollination and thus might avoid monitoring measures on the non-GM field or on the truck delivering the non-GM commodity to the elevator.

[16] These hypotheses do not alter general conclusions but prevent us from providing quantitative estimation.

Generally speaking, results obtained in different regions demonstrated that coexistence costs depend on the agricultural context (landscapes, cropping systems, climate, practices), the share of GM crop (maize or oilseed rape) in the Agricultural Used Area (AUA) and the willingness of GM and non-GM farmers to cooperate.

Uniform non-flexible coexistence rules, such as standardized large isolation distance requirements between GM and non-GM crops, while providing a margin factor for adventitious presence of GM in non-GM production, might impose a severe burden on GM crop production in the European regions investigated in this study. Indeed, cross-pollination highly depends on structural factors like landscape field patterns, agronomic practices and climatic conditions and, in most cases, small isolation distances would be sufficient to meet the official threshold of 0.9%. Large uniform isolation distances, as implemented by most European countries, are not flexible and, therefore, not proportional to the actual risk of adventitious presence.

In addition, large and/or fixed isolation distance requirements may lead to a domino-effect[17]. so that farmers would have few, if any, fields complying with these isolation distances and would be unable to cultivate GM crops. This domino-effect can also occur with smaller fixed isolation distances in areas with small fields and a high density of cropping with the same crops. This effect is particularly important at low levels of GM adoption as the probability of a GM field of having a non-GM field nearby is higher even though the overall cross-pollination potential is lower. Conversely, the domino-effect would be less of a problem for higher adoption rates of GM crops. The domino-effect exacerbates the non-proportionality of wide isolation distances by reducing GM crop planting options in the landscape and raising opportunity costs for GM crop adopters.

Flexible measures based on buffer zones or discard zones may require compensation of loss of income by non-GM farmers, whenever and wherever it occurs, but lead to lower overall coexistence costs and are proportional to the incentives for coexistence and, consequently, less counterproductive for European agriculture. However, they require a high level of coordination between farmers and hence assume that farmers will cooperate and accept additional transaction costs and financial risks. Under these conditions, flexible measures lead to a natural minimization of coexistence costs as farmers will negotiate the measures that reduce overall costs and reflect their incentives for coexistence in the long-run.

GM seed price premium had no significant effect on costs of coexistence, as non-GM seed price might also increase, while coexistence costs increased with the Identity Preservation (IP) price premium, due to factors such as greater demand for non-GM crops. The benefits of GM crop adoption are generally higher than the costs of coexistence (transaction costs not considered). It was concluded that GM crop adoption is not an issue of costs of compliance to coexistence measures but rather one of the incentives for adopting or rejecting the technology. From the economic point of view, coexistence is only a subject of concern when there is significant preference for non-GM crops to GM crops.

As far as flexible coexistence are considered (buffer or discard zones), the average per-hectare coexistence management costs, although variable, were relatively independent from the GM adoption rate in moderately dense areas such as Aragon (maize) or Scotland (oilseed rape). There are, however, large differences regarding the monitoring costs which

---

[17] The domino-effect is a dynamic spill-over effect of farmer decisions induced by enforcing wide isolation distances on potential GM crop adopters. It consists in the iterative process of farmers switching their planting intentions from 'GM' to 'IP' crops to comply with isolation distances and hereby restricting planting options of neighbouring farmers.

are related to GM crop adoption rates: the higher the GM adoption rate, the lower the additional per-ha costs of coexistence[18].

In Alsace, SIGMEA was able to test the impact of the agricultural structure on coexistence costs by comparing a region with small farms and small field sizes (Heiwiller) with a region with medium-sized farms and larger field sizes (Ensisheim). The coexistence costs are higher in those regions with a smaller scale of agricultural structures (fields, farms). This is due to higher transaction costs on the one hand and a higher share of monitoring costs and discard zone areas on the non-GM maize area on the other hand. The latter leads to higher compensation costs for income forgone by the non-GM farmers.

Crucial to the evaluation of the coexistence costs for non-GM farmers producing for the IP market are the perceived effectiveness of the implemented coexistence measures, the non-GM farmer's willingness to take the risk of non-compliance with IP market conditions and the non-GM farmer's trust in liability or insurance procedures in the case of system failure. Monitoring can be a significant cost for non-GM farmers so that, in some situations, overall coexistence costs of non-GM farmers can be decreased by increasing discard zone sizes as this can result in lower monitoring requirements and costs. However, in some cases, the respective discard zone area required exceeds up to 99% of the envisaged non-GM maize area. As a consequence of these large discard zone areas, IP maize production in those cases is impossible.

Flexible coexistence regimes without discard zones would lift spatial constraints but is likely to increase the number of downgraded non-GM maize lots (fields not complying with the official threshold or any other IP requirements). Such regimes may be economically viable if the assumed insurance fee (e.g., 14 €/ha used in our work) could cover the compensation of non-GM farmers for downgraded IP maize produce. This is more likely to occur for small adoption rates. Nevertheless, such flexible coexistence regimes would not work at all in situations where GM-free production is required. As a consequence, downstream supply chain actors who demand pure GM-free IP produce might not be willing to accept deliveries from non-GM farmers in regions with flexible coexistence regimes. Thus, even though GM farmers would be able to compensate potential income forgone of the IP maize farmers with the insurance, those non-GM farmers might be excluded from IP maize market channels. Coexistence in this case would thus be impossible due to market exclusion of the non-GM farmers.

Finally, we addressed transboundary issues by analyzing the situation of maize farmers cultivating land along the border between France and Switzerland and considering that GM varieties were sown in France while GM cultivation was not permitted in Switzerland due to a five year moratorium. Swiss fields cultivated along the borders would be affected by cross-pollination with GM maize grown in the neighbouring country. In this case, low thresholds could not be met without implementing a strategy for coexistence in the non-GM growing country which may lead to legal issues. Growing non-GM maize in the border region would require exchange of information (location of GM crops, coexistence strategies, liability and thresholds) and additional measures to avoid admixture of GM and non-GM crops.

In summary, these SIGMEA studies demonstrate that the economics and appropriateness of different measures are mainly determined by the spatial and temporal patterns of fields and crops. This indicates that coexistence management measures should be as flexible as possible and based on local information on field characteristics whereas regional and national governance provides only general guidelines and rules.

---

[18] Monitoring costs of non-GM fields might increase but would be supported by a larger GM acreage.

SIGMEA has produced the first large-scale empirically based estimation of the economic impact of a GM crop for EU farmers.

Currently the only GM crop authorised for commercial cultivation in the EU is Bt maize, resistant to certain stem borer pests. Spain has the largest surface of Bt maize in the EU and over 9 years of commercial experience in cultivation. The Spanish case presented an opportunity to study *ex-post* the agronomic and economic performance of a GM crop in the EU. Analyses of GM crop impacts on farm economies are usually based on surveys of farmers cultivating GM crops under commercial conditions. A face-to-face survey was conducted among Spanish commercial maize farmers with the aim both of obtaining data on the agronomic and economic performance of *Bt* maize during three growing seasons (2002-2004) and of comparing the socioeconomic profile of farmers who adopted *Bt* maize versus those who did not. The survey was conducted in the three leading *Bt* maize-growing regions (Aragon, Catalonia and Castilla-La Mancha), which accounted for approximately 90% of the *Bt* corn-growing area in Spain in 2006. A province was selected within each region based on the importance of maize cultivation and the presence of farmers growing *Bt* maize (the provinces of Zaragoza in Aragon, Albacete in Castilla-La Mancha and Lleida in Catalonia).

Survey results found that *Bt* maize, like other pest-control technologies, produced variable impacts on maize yields in different provinces, ranging from neutral to 11.8% yield increase. The regional variability depends mainly on local variations of pest pressure and damage. Yield gains for growers of *Bt* maize were translated into revenue increase since no differences were found in the price paid to farmers for *Bt* or conventional maize. Regarding production costs, Bt maize growers paid more for the seeds than conventional growers, but had reduced insecticide use and costs. On average, growers of conventional maize applied 0.86 insecticide treatments/year to control borers and other insects, versus 0.32 treatments/year applied by Bt maize growers. All things considered, the impact of *Bt* maize adoption on gross margin obtained by farmers in different provinces ranged from neutral to € 122/ha per annum. In the survey, the reason most quoted by farmers for adopting Bt maize was “lowering the risk of maize borer damage” followed by “obtaining higher yields”.

Finally, the survey compared the socio-economic profiles of farmers adopting or not *Bt* maize varieties. No differences were found for the two groups of farmers for variables such as land ownership, farm size, experience as maize grower, education or training. The conclusion is that the differences in yields and gross margin are therefore attributable to the adoption of Bt maize varieties.

SIGMEA has also produced the largest survey to estimate *ex ante* the potential adoption by farmers of three GM crops not yet authorized in the EU but widely grown elsewhere: Herbicide Tolerant (HT) oilseed rape, HT maize and Bt/HT maize (combining herbicide tolerance and insect resistance). It has also looked at the impact of proposed coexistence measures on the willingness of farmers to adopt GM crops. A face-to-face survey of 1214 European farmers with a questionnaire specifically designed for this study was the main source of data. Germany, France, Spain, Hungary, United Kingdom and Czech Republic were chosen as countries to be studied. All these countries are major producers of maize and/or oilseed rape.

Analyses of farmers’ responses show that there is high potential adoption of HT oilseed rape and HT maize, as well as Bt/HT maize. On average, forty-one percent of the farmers surveyed in the six countries are prepared to plant these GM crops. This figure nevertheless depends to a large extent on the coexistence measures put in place by EU member states. Measures strongly affecting potential adoption of GM crops are the obligation to pay compensation to nearby farms in case of unintended admixture, a GMO tax or the introduction of an insurance mechanism to cover dissemination risks. In addition, if mandatory separation distances for GM crops are excessive, then many farmers would not adopt GM crops.

Table 2: Potential adoption of GM crops by EU farmers: results of a survey carried out in 2007 in 5 countries.

Trait/Crop	**Country**	**(1)** **Likely+very-likely %**	**(2) Unlikely + Very-unlikely %**	**Ratio** ***(1)/(2)***
HT rapeseed	Germany	53,4	31,7	1,68
	United Kingdom	44,0	25,5	1,73
	Czech Republic	43,9	28,1	1,56
HT maize	Spain	36,5	38,5	0,95
	France	37,6	33,7	1,12
	Hungary	38,0	38,0	1,00
Bt/HT maize	Spain	48,3	35,0	1,38
	France	46,5	28,7	1,62
	Hungary	25,3	57,6	0,44
	Total average	**41,5**	**35,2**	**1,18**

A framework for designing multi-attribute decision-support systems has been proposed

Genetically modified (GM) crops have become an option in modern agriculture but they also raise concerns about their ecological and economic impacts. Decisions about GM crops are complex and call for decision support. SIGMEA has been examining decision tools which would help stakeholders and decision-makers to better understand the implications of growing GM crops.

A first model, the so-called "Grignon" model, is a qualitative multi-attribute model for the assessment of ecological and economic impacts at a farm level of GM and non-GM maize crops which was developed together with the UE ECOGEN research project. The model is applied for one agricultural season. This is an ex-ante model developed according to multi-attribute decision tree methodology. In this model, cropping systems are defined by four groups of features: (1) crop sub-type, (2) regional and farm-level context, (3) crop protection and crop management strategies, and (4) expected characteristics of the harvest. The impact assessment of cropping systems is based on four groups of ecological and two groups of economic indicators: biodiversity, soil biodiversity, water quality, greenhouse gasses, variable costs and production value. The evaluation of cropping systems is governed by expert-defined rules.

The "Grignon" model has been used to assess hypothetical and real maize-based cropping systems. For each system, we are able to obtain a qualitative overall assessment together with its 'profile', i.e., its performances for the main economic and ecological attributes.

Moreover, one can ‘drill-down’ into lower levels of the model to identify the most sensitive components.

It represents a practical means encapsulating a complex system as it integrates findings of different specific disciplines, such as agronomy, biology, ecology and economics (although it cannot capture specific details of any of these disciplines), and provides a general overview to the assessment of cropping systems which can then easily support discussion among experts and stakeholders.

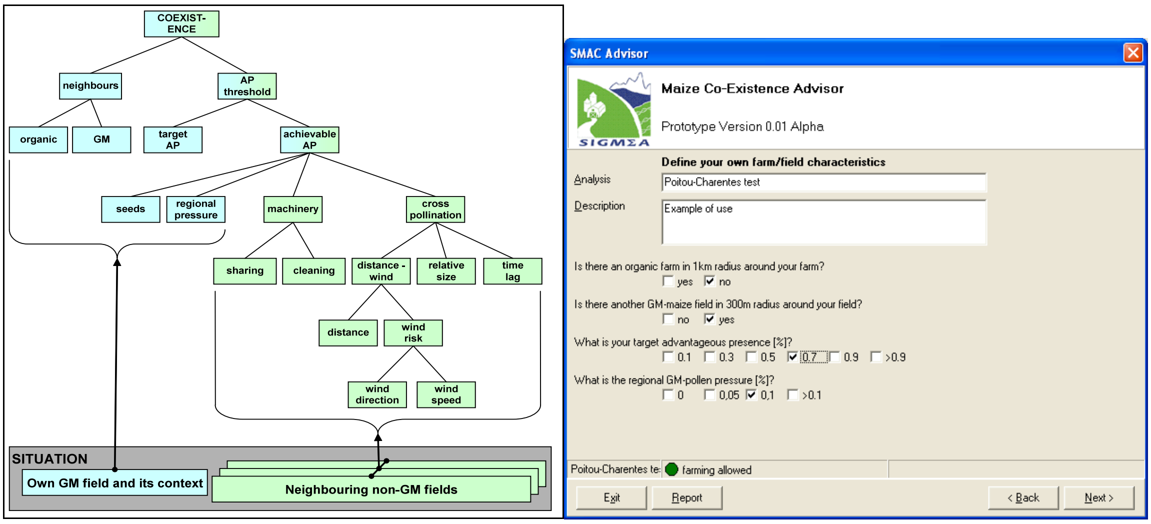
The issue of coexistence was also considered: is it possible, under which conditions and to which extent, to grow both GM and non-GM (conventional) crops simultaneously or in close proximity and ensure that non-GM crops would meet a targeted threshold of adventitious presence? As stated above, the answer can be extremely complex as coexistence involves many variable factors, which are difficult to assess, predict and control such as pollen flow, volunteers, feral plants, mixing during harvesting, transport, storage and processing, human error, and accidents. The LandFlow-Gene platform has been designed to assess gene flow at the agricultural landscape level. At present LandFlow-Gene cannot be used on a real-time basis by end-users as quite a lot of data describing landscapes, climate and practices are required. To allow farmers to carry out a preliminary in-field diagnosis, SIGMEA developed a decision-support tool called *SMAC Advisor*, which is aimed at providing advice to farmers and other decision-makers (administrative workers, policy makers) who want to assess the achievable level of maize coexistence on a given field and in a given agricultural environment. The assessment is based on a qualitative multi-attribute decision-support model, which was constructed from two sources: (1) MAPOD® gene-flow simulations under constrated situations and (2) expert-provided rules.

SMAC Advisor formulates the decision problem as follows:

Suppose a farmer wants to start growing GM maize on field F. In the neighbourhood, there are some other fields, E_1, E_2, …, E_n, on which this or other farmers grow (or want to grow) non-GM maize. Then, the question is: to what extent will the plants grown on F genetically interfere with the plants on E’s? Will this interference be small enough to allow coexistence? The “interference” between plants is expressed and measured in terms of *adventitious presence* (AP). AP refers to the unintentional and incidental commingling of trace amounts of one type of seed, grain or food product with another. EU regulations have introduced a 0.9 % labelling threshold for the AP of GM material in non-GM products (Regulation 2003/1830/EC). Thus, in order to approve the coexistence between GM and non-GM crops, we usually require that the achieved AP is 0.9 % or less. Now, some supply chains may require lower levels of AP (e.g., organic farming). In SMAC Advisor, the target threshold is a user-defined parameter.

Basically, SMAC Advisor requires basic information from the user about the: (1) emitting field F, (2) neighbouring fields E_1, E_2, …, E_n, (3) relation between F and each E_i in terms of distance, relative size, prevalent wind direction, etc., (4) type and characteristics of used seeds, (5) environmental characteristics (e.g., background GM pollen pressure), and (6) use of machinery (e.g., sharing with other farmers). All these elements can easily be provided by the end-user (e.g., farmers) through a user-friendly interface (Figure C).

On this basis and through a multi-attribute decision tree (Figure D), SMAC Advisor determines the *achievable AP*, that is, the expected level of GM impurities in harvests of the neighbouring fields, and compares it with the required *target AP*, which is provided by the user. SMAC Advisor completes the analysis giving one of the following “colour-coded” *recommendations*: (1) “Green”: GM farming allowed or possible, (2) “Red”: GM farming disallowed, (3) “Yellow”: coexistence is possibly achievable but further risk assessment is needed, and (4) “Orange”: the target AP is currently not achievable, continue assessing additional coexistence measures.



Maize Co-Existence Advisor

Prototype Version 0.01 Alpha

Define your own farm/field characteristics

Analysis: Poitou-Charentes test

Description: Example of use

Is there an organic farm in 1km radius around your farm?
☐ yes ☑ no

Is there another GM-maize field in 300m radius around your field?
☐ no ☑ yes

What is your target advantageous presence [%]?
☐ 0.1 ☐ 0.3 ☐ 0.5 ☑ 0.7 ☐ 0.9 ☐ >0.9

What is the regional GM-pollen pressure [%]?
☐ 0 ☐ 0,05 ☑ 0,1 ☐ >0.1

Poitou-Charentes te: ● farming allowed

Figure D. Description of SMAC Advisor: hierarchical attribute structure and user interface.

On-site novel methods for GMO detection have been designed

A pre-harvest method to estimate the GM content of conventional maize fields, employing a duplex RT-PCR detection and quantification assay for MON810 for use on the Cepheid SmartCyclerII on-site instrument as a model, was developed and validated through an international ring-trial. Assay performance met minimum requirements as considered by the European network of GMO Laboratories (ENGL). Complimentary to this, two field-level sampling procedures have been further investigated with suggestions for practical implementation. Together, both elements (method and sampling procedure) constitute the basis for a strategic "prototype" on-site decision tool for assessing GM adventitious presence pre-harvest. In addition, a protein based strip-test, based on a commercial kit, was also validated in-house for use in a semi-quantitative capacity against maize, and in support of the RT-PCR method.

In addition, an in-house validated qualitative strip-test for Round-up Ready oilseed rape, originally commercialised for use with soybean, was shown to function adequately.

As it was considered more appropriate to make such method information available in a more established and purpose built database for public access, the GMOs Method Database hosted by the Joint Research Centre's Institute for Health and Consumer Protection (IHCP), Ispra, Italy (http://biotech.jrc.it/home/ict/methodsdatabase.htm#Database) has been selected to host the these details. For copyright reasons, this will be finalised once the methods have been published in a peer reviewed format.

With respect to the maize field-level sampling schemes, as part of the delivery of the prototype pre-harvest predictive tool, a number of important conclusions from both studies towards accurate estimation of field-level GM presence highlight the necessisty to sample kernels from cobs on many plants, and not from single plants. In this way the probability distribution of cross-pollination is also better sampled. Therefore it is better to sample a few kernels from many cobs, rather than many kernels from a few cobs, although the former is more problematic in practice – it would be less prone to plant-to-plant variation and sampling error. In addition, further investigation of optimal in-field sampling schemes should be performed to take into account the intra-field distribution of cross-pollination (boundaries have a higher cross-pollination level).

Monitoring issues for EU including recommendations for regulation, relevant biological indicators, sampling and detection methods

A coherent structure for GMO monitoring in Europe is still under development. This refers not only to the central level of European institutions but refers also to member state and to the regional regulatory levels. In many member states, biodiversity assessments are not implemented in ways that provide results relevant to GMOs. Standard environmental and agricultural monitoring are not always appropriate for capturing the relevant effects and associating them with GMOs. Methods require further development which is is still "in progress". One reason for slower implementation may be the regulatory statement that the notifiers are held responsible for this task in financial terms. Notifiers have to cover the relevant expenses either by executing the required tasks or compensating for required activities by the authorities. It seems questionable whether this is appropriate for GMO monitoring as environmental monitoring is also a sovereign responsibility.

The molecular analytical effort of the Central Reference Laboratory together with the European Network of GMO Laboratories ENGL are primarily focused on GMOs. These are the most comprehensive structures established for GMO assessment and are largely institutionalised by the EU as a precondition for efficient regulation. This is reasonable to fulfil sovereign tasks of identifying approved and unapproved GMO presence in a range of imported and manufactured products. A similar network is required for the assessment of anticipated and unanticipated long-term and combinatory effects of GMOs. The necessity of sovereign engagement becomes also apparent in the context of data collation and synthesis requirements. Evaluating completeness, consistency and quality standards of measurements and to draw conclusions has to be done at an administrative level. Therefore, it appears useful that the European Union as well as the member states expand their initiatives in this field – to provide basic data, model-supported synthesis capacities and decision making. To develop such regulatory steps competent authorities will need to be well informed on the scientific rationales for monitoring and prepared to integrate monitoring activities both nationally and internationally.

The current regulatory regimes of EU and member states, liability and redress issues have been analyzed and recommendations have been made.

Following the research carried out on liability and redress issues and analysis of scenarios, the following conclusions are drawn for the regulatory regime in the EU.

Does GMO pose novel problems for the law?

There are no novel problems posed at the present time by GMO for the questions of liability and redress. The sorts of harms, the causation issues and contributory issues can be seen in a number of analogous risk activities (e.g., asbestos injuries, smoking related illness, drug regulation, product liability, and food production). These analogous situations have been met by different legal solutions both at the national, regional and international level. However, it could be that long-term difficulties emerge that are not foreseen at the present time.

Are there any problems which make particular established legal tools unsuitable as options for the GMO problems?

There is a range of established legal tools available to regulate GMOs. Civil regimes, insurance-based regimes, and compensation-based state regimes were all studied and none shows any particular technical problems. There is, of course, the question for the insurance model of whether a market can be established to make this a viable regime.

Is there any particular regime that suggests itself as appropriate to the GMO issue?

There is no particular regime that stands out as appropriate for use in the GMO issue. However, this is not because all the models are equally appropriate and attractive. Rather it is because of a number of significant external factors which were considered in drawing conclusions for this report.

Recommendations

- The first and only concrete recommendation that can be made is that the trans-border issues relating to GMO make the desirability of an EU-wide single legal regime very strong. This would eliminate costly conflict of laws problems between member states. This would, however, require a degree of agreement over the desirability of GMOs in the Union, which on current form is unlikely.

- Whereas a regime could be entirely no-fault based, there could be arguments for the application of the polluter pays principle where this would be seen to act as a deterrence against deliberate harmful actions, recklessness, negligence and carelessness. It could also raise the industry standards. However, the polluter may not be able to pay, requiring a mandatory insurance (with enforcement). This in turn depends upon the viability of a market for insurance (i.e. a financial return for the insurance industry). The question of deterrence may be better served through criminal sanctions and a blanket, no-fault compensation scheme.

- The question of responsibility clearly needs resolution before the choice of regulatory regime can be set. It would seem logical that those who encourage the development of the technology, be it state or consumer, actively or passively, bear levels of responsibility for the consequences of those choices. This requires consideration in relation to the farmer and producer as agent of the state and consumer (with the analogous issues of liability where the individuals outside the terms of the agency – e.g. in this case, where the farmer acts deliberately or recklessly).

- There is the over-riding question of who actually pays. There is the question of how far that liability (fines, etc.) are passed down the chain to the last individual (consumer) who cannot pass on costs. There is no guarantee that the added costs of a system requiring the investigation of proof and blame will be more efficient than a compensation scheme.

- Equally, there is the question in a taxation system of why someone who does not want to participate in the new technology must pay for the liability and redress issues caused by such a technology.

- So the overall choices of regulatory regime concern the causation, foreseeability, responsibility, and participation. These must be considered in relation to the cost and practicality of the scheme. The great number of harmful, risky activities in modern society produce a vast range of analogous situations which provide evidence that any legal model could be applied.

- There is also a broader question of why GMO is taken in isolation and treated as a special case. There are harms and issues concerning liability and redress in non-GMO agriculture, organic and non-organic, and yet the GMO is taken for special consideration outside the whole structure of agriculture. There is a very strong argument for taking the GMO debate within the broader agricultural questions.

Recommendations for the decision-making processes relating to the market release of GM crops under progress can be derived from SIGMEA outcomes

Although gene flow is a common phenomenon for crop species, its implications for Genetically Modified Plants have raised new concerns. Undesirable effects related to gene flow may result in ecological or agronomic considerations (persistence of resistant volunteers, creation of new weeds, multiple resistances) as well as commercial considerations (unintended presence of GMOs in conventional crop production affecting its competitiveness in the marketplace). The coexistence between different types of crops is an important issue and has to be addressed once GM crops are approved in the EU. The European Union has issued guidelines designed to allow for the coexistence of various kinds of agriculture in support of its policy that "farmers should be able to cultivate freely the agricultural crops they choose, be it GM, conventional or organic" (Recommendation 2003/556/EC). New GMO regulations have been introduced as a basis for Member states to develop appropriate coexistence and traceability measures for delivery of food and feedstuffs complying with the labelling thresholds.

SIGMEA has produced a practical toolbox for addressing GM impacts in agriculture:

1. A unique database including more than 100 datasets on geneflow and ecological impacts which may inform decision-makers on factors driving gene flow at the landscape level and on the variability of such processes across Europe, help regulators to set up coexistence measures at National levels as well as help scientists to identify further research priorities in that area.

2. LandSFACTS is a user-friendly windows-based software to simulate crop allocation to fields by integrating typical crop rotations and crop spatio-temporal arrangements within agricultural landscapes and could be used for a practical implementation of coexistence measures

3. The generic gene flow platform LandFlow-Gene, including validated rapeseed and maize modules and interfaced with the landscape generator LandSFACTS and GIS softwares, is now available as a prototype. It is used to support regional case studies analysis and discussion about scenarios. This platform could be extended to other crops to provide a general framework for informing coexistence in all cropping systems of Europe.

4. A user-friendly decision-support system to assess maize coexistence feasibility at the field level was designed.

5. Structural and organisational factors affecting coexistence in practice have been identified and strategies for managing coexistence at the regional level have been proposed;

6. A comprehensive overview of monitoring and legal issues has been provided but, due to the delay in implementing regulations in most member states and the low development of commercial GM cropping in Europe, only general recommendations have been made.

Altogether, these tools and outcomes can be combined to assess coexistence at various spatial scales (field, farm or region) and various decision-making levels (farmers, elevators, member states, EU). Depending on the decision problem and the amount of information available, various SIGMEA tools can be used.

SIGMEA findings make it possible to address issues such as "what will happen, in terms of gene flow, if a particular GM organism is introduced into a particular European region?" and “how can crops be deployed at the landscape level so as to maintain the adventitious presence of GMOs in conventional crops within the legal thresholds, or any specific market-driven requirements?”.

The outcome of both field and modelling studies carried out in SIGMEA is that best practices for coexistence are highly variable and depend on local characteristics, crop practices, environments as well as farmer strategies and preferences, and that the feasibility of coexistence directly depends on the targeted threshold.

Based on regional case studies findings, contrasting global coexistence scenarios may be defined by considering different regulation approaches:

- A “bottom-up” approach, which would let the private actors (collectors, farmers) free to choose the best way to achieve coexistence guidelines and to meet regulatory or market-based threshold requirements;
- A “top-down” approach, based on the strong intervention of public authorities with the implementation of compulsory uniform measures (e.g., isolation distances);
- and a “third way” approach, which provides a focused response of authorities to lift some constraints on private actors.

It has been stressed that coexistence regime based on “uniform isolation distances”, as implemented so far in several member states, are not optimal, not proportional and may lead to unnecessary additional costs or render coexistence impossible.

SIGMEA thus recommends that coexistence measures should be as flexible as possible. And depend on local climatic, agronomic and environmental factors. Such an approach would lead to more cost-efficient measures. However the current regulatory framework to support such an approach is still to be developed.

SIGMEA has developed tools to support such an approach. Predictive gene flow models are now available (currently only for maize and oilseed rape but easily extendable to other crops). These can help decision-makers assess the feasibility of coexistence at the field, farm and silo level for the various targeted thresholds under various environmental and agronomic conditions. In addition simple decision-support tools, like SMAC Advisor can be used by farmers or advisors who would like to quickly assess coexistence feasibility using limited amounts of information at a local field level.

SIGMEA is providing the scientific community as well as decision-makers with adequate information about gene flow and its implications in terms of co-existence.

To date, SIGMEA partners have published around 70 refereed papers on issues associated with gene flow, coexistence and gene detection and a further 40 papers have been submitted for publication, many of them already accepted. In addition, SIGMEA contributed to book chapters on GMO issues, European and National government reports and public debates.

SIGMEA was very directly involved in the organization of the two last conferences on coexistence (GMCC05 in Montpellier – www.gmcc05.com – and GMCC07 in Seville – http://teamwork.intbase.com/0703_03/new_index.php). At GMCC07 there were 17 oral presentations by SIGMEA partners including papers summarising scientific knowledge on gene flow in maize, oilseed rape and sugar beet from the SIGMEA data sets and other papers reporting findings from SIGMEA studies. There were also 24 poster presentations.

13 PhD theses and 5 Masters were submitted during the period of the project. SIGMEA partners were also involved in events related to communication to extension services and farmers as well as in public debates, press articles, radio/TV interviews.

This literature review is part of the National Research Programme NRP 59 “Benefits and Risks of the Deliberate Release of Genetically Modified Plants”. It is one of a total of three such reviews, which are:

- Medical issues related to genetically modified plants of relevance to Switzerland
- Genetically modified crop production: social sciences, agricultural economics, and costs and benefits of coexistence
- Synthesis and overview studies to evaluate existing research and knowledge on biological issues on GM plants of relevance to Swiss environments

They aim to distil relevant scientific data from the results of international studies on GMP that could help to shape future research and decision-making processes in Switzerland.

vdf
www.vdf.ethz.ch
verlag@vdf.ethz.ch

ISBN 978-3-7281-3498-1 Print version
ISBN 978-3-7281-3499-8 eBook
DOI-Nr. 10.3218/3499-8

Benefits and Risks of the Deliberate Release of Genetically Modified Plants
National Research Programme NRP 59