Ecological impacts of genetically modified crops

**Experiences from ten years of experimental field research
and commercial cultivation**

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Editorial

The Swiss Expert Committee for Biosafety SECB advises on issues related to the protection of people and the environment in the areas of biotechnology and gene technology. Consequently, the SECB counsels the authorities on permit applications for field trials and commercial cultivation of genetically modified (GM) crops and issues statements on safety aspects of GM crops.

The SECB has appointed the Agroscope Reckenholz-Tänikon Research Station ART to review the available scientific data on ecological effects of commercially grown GM crops to enable the Committee to apply the results obtained from the worldwide experience with GM crops as a basis for its decision-making process. The present study focuses on insect-resistant maize and herbicide-tolerant soybean and oilseed rape, respectively, representing three of the major GM crops of significance for Swiss agriculture, and their possible impact on the environment. Peer-reviewed journals, scientific books and reports from international organizations have been the main source of data for the study.

We believe that the study offers a science-based and broad overview of the worldwide situation of possible impacts of GM crops on the environment. We regret, however, that most of the data originate from large-scale field trials rather than from commercial cultivation. This lack of data from commercial cultivation is mostly due to the fact that legal requirements for a post-market monitoring of GM crops are missing in the major GM crop-producing countries. Furthermore, the conventional farming practices in those countries differ largely from the integrated-production system mainly applied in Switzerland, which makes the transferability of data difficult, especially when internationally recognized baselines for the comparison of environmental effects of GM crops in the context of modern agricultural systems are not available.

Some committee members would have preferred a stronger focus on remaining uncertainties and knowledge gaps, as well as on the controversial discussions among scientists concerning the interpretation of scientific data. Therefore, the study does not represent the position of all committee members. A minority of the committee members dissents with the conclusion that the data available up to now do not provide any scientific evidence for harm caused to the environment by commercial cultivation of GM crops.

Nevertheless, we believe that the Committee will profit from the worldwide experience drawn from the cultivation of GM crops reviewed in this study when assessing the risk of field trials with GM crops, taking into account the precautionary approach and a sustainable agricultural development in Switzerland. We hope that this study contributes to a sound discussion on benefits and risks of GM crops.

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Summary

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The global area planted with genetically modified (GM) crops has consistently increased each year since GM crops were first commercially cultivated in 1996 reaching 90 million hectares in 2005. GM crops are currently grown by 8.5 million farmers in 21 countries, where 90% of the farmers using the GM technology live in developing countries. Five countries (USA, Argentina, Brazil, Canada and China) are growing nearly 95% of the total area of these crops and there are four main GM crops that are grown worldwide. Soybean is the principal GM crop occupying most of the global area, followed by maize, cotton and oilseed rape. Herbicide tolerance is the dominant trait that is deployed in all four crops, while maize and cotton are the only two insect resistant GM crops commercialized. Concerns have been raised that the commercial cultivation of GM crops could result in adverse effects on the environment. Agroscope Reckenholz-Tänikon Research Station ART was commissioned by the Swiss Expert Committee for Biosafety to review the scientific knowledge on environmental impacts of GM crops deriving from ten years of worldwide experimental field research and commercial culti-vation. The sources of information included peer-reviewed scientific journals, scientific books, reports from countries with extensive GM crop cultivation, as well as reports from international organizations. For some of the questions addressed only limited information was available from commercial cultivation. Therefore most chapters of the study include to some extent scientific data deriving from large-scale experimental field research. The authors recognize that results from large-scale cultivation systems, as often characteristic in the countries growing GM crops, have to be transferred with care to small-scale agri-cultural systems like in Switzerland. However, we believe that the worldwide scientific knowledge and the existing practical experiences should be taken into account for future decision making when discussing potential risks of field releases of GM crops in Switzer-land.

The safety of GM crops is generally assessed more intensely than that of convention-ally bred crops because, in addition to the selection process performed during classical breeding, a thorough pre-market risk assessment of potential unwanted effects of the GM crop on the environment is a prerequisite to obtain permission to market any GM crop variety. The risks of GM crops for the environment, and especially for biodiversity, have been extensively assessed worldwide during the past ten years of commercial cultivation of GM crops. Consequently, substantial scientific data on environmental effects of the currently commercialized GM crops is available today, and will further be obtained given that several research programmes are underway in a number of coun-tries. The data available so far provides no scientific evidence that the commercial culti-vation of GM crops has caused environmental harm. Nevertheless, a number of issues related to the interpretation of scientific data on effects of GM crops on the environment are debated controversially. To a certain extent, this is due to the inherent fact that scien-tific data is always characterized by uncertainties, and that predictions on potential long-

term or cumulative effects are difficult. Uncertainties can either be related to the circumstance that there is not yet a sufficient data basis provided for an assessment of consequences (the "unknown"), or to the fact that the questions to solve are out of reach for scientific methods (the "unknowable"). Although some might argue that experience and solid scientific knowledge are still lacking, the debate is generally not purely due to a lack of scientific data, but more to an ambiguous interpretations of what is considered an ecologically relevant effect of GM crops. The interpretation of study results is thereby often challenged by the absence of a defined baseline for the comparison of environmental effects of GM crops in the context of modern agricultural systems. There is thus a need to develop scientific criteria for the evaluation of effects of GM crops on the environment in order to assist regulatory authorities when deciding whether environmental effects of GM crops are considered as relevant. In the present study, the effects of GM crop cultivation on the environment are discussed considering the impacts caused by cultivation practices of modern agricultural systems. Independent from the use of GM crops, modern agricultural systems have profound impacts on all environmental resources, including negative impacts on biodiversity. Several changes in the management of agricultural land over the last century have resulted in a decline in the biodiversity within agro-ecosystems.

There are concerns that insect-resistant GM crops expressing Cry-proteins from *Bacillus thuringiensis* (*Bt*) could harm organisms other than the pest(s) targeted by the toxin. The published large-scale studies in *Bt*-crops assessing possible non-target effects on arthropods have only revealed subtle shifts in the arthropod community, which can be explained by a lack of the target pest resulting from the effective control by the *Bt*-crops. No adverse effects on non-target natural enemies resulting from direct toxicity of the expressed *Bt*-toxins have so far been observed in laboratory studies and in the field. There is evidence that the *Bt*-crops grown today are more target-specific and have fewer side effects on non-target organisms than most current insecticides used. The adoption of *Bt*-maize has resulted in only modest reductions in insecticide applications due to the small area of conventional maize treated with insecticides against the European Corn Borer. The commercial cultivation of *Bt*-cotton, in contrast, has resulted both in a substantial reduction in quantity and in number of insecticide applications. In addition to direct environmental benefits such as fewer non-target effects and reduced pesticide inputs in water, demonstrable health benefits have been documented for farm workers in developing countries due to less chemical insecticide spraying in *Bt*-cotton.

Similarly to non-target effects above ground, concerns were raised that *Bt*-crops could have effects on soil organisms. *Bt*-toxins enter the soil system primarily via root exudation and via plant remains after harvest. Both degradation and inactivation of the *Bt*-toxin vary, depending on parameters such as temperature and soil type. The initial degradation of the toxin is rapid, while a low percentage (< 2%) may remain in the soil ecosystem following one growing season. *Bt*-toxins have been shown to bind to clay and humic acid compounds, however, no accumulation of toxins has been observed after several years of cultivation of *Bt*-crops. Population sizes and community structure of soil organism are subject to both high natural seasonal variation and to variations caused by the agricultural system (soil type, plant age, crops, cultivars and crop rotation). Neither laboratory nor field studies have shown lethal or sublethal effects of *Bt*-toxins on non-target soil organisms such as earthworms, collembola, mites, woodlice or nematodes. Some differences in total numbers and community structure have been described for microorganisms. The ecological significance of the observed differences is not clear. Because most studies have not assessed the natural variation occurring in agricultural

systems, it is generally difficult to establish whether the differences between *Bt*- and non-*Bt*-crops were exceeding this variation. The only study considering natural variation suggests that observed effects lie within this variation, and that the differences between conventional cultivars outweigh the observed influences of *Bt*-crops.

There is general scientific agreement that gene flow from GM crops to sexually compatible wild relatives can occur. Experimental studies have shown that GM crops are capable of spontaneously mating with wild relatives, however at rates in the order of what would be expected for non-transgenic crops. Few studies have shown that GM herbicide tolerant (GMHT) oilseed rape (*Brassica napus*) can form F1 hybrids with wild turnip (*Brassica rapa*) at low frequency under natural conditions. Questions remain whether these transgenes would cause ecologically relevant changes in recipient plant populations. Although there is a low probability that increased weediness due to gene flow could occur, it is unlikely that GMHT weeds would create greater agricultural problems than conventional weeds. Farmers can generally choose among several herbicides for the cultivation of a given crop and they have further a set of options within a crop rotation to control or manage weeds.

In natural habitats, no long-term introgression of transgenes into wild plant populations leading to the extinction of any wild plant taxa has been observed to date. Transgenes conferring herbicide tolerance are unlikely to confer a benefit in natural habitats because these genes are selectively neutral in natural environments, whereas insect resistance genes could increase fitness if pests contribute to the control of natural plant populations.

Despite the concern of GM crops invading natural habitats was brought up early in the discussion on potential environmental risk related to the release of GM crops, it seems that modern crop varieties generally stay domesticated. There is no evidence at present that the extensive cultivation of GMHT oilseed rape over several years in Western Canada has resulted in a widespread dispersal of volunteer oilseed rape carrying herbicide-tolerance traits. Although one study found triple-herbicide resistant, and another study reported double-herbicide resistant oilseed rape volunteers in Western Canada, the general lack of reported multiple-resistant volunteers suggests that these volunteers are being controlled by chemical and non-chemical management strategies, and are therefore not an agronomic concern to most farmers. Furthermore, there is currently no evidence that GMHT oilseed rape has become feral and has invaded natural habitats.

Impacts of GM crops on pest and weed management practices and their potential ecological consequences are usually difficult to assess. They are generally influenced by many interacting factors and do often only show up after an extended period of time. Numerous weed species have evolved resistance to a number of herbicides long before the introduction of GMHT crops. The experiences available from regions growing GMHT crops on a large-scale confirm that the development of herbicide-resistances in weeds is not primarily a question of genetic modification, but of the crop- and herbicide management applied by farmers. Despite the extensive cultivation of GMHT oilseed rape in Canada, no weed species has so far been observed being tolerant to the herbicides glyphosate and glufosinate. In continuously cultivated GMHT soybeans in the United States, in contrast, many fields have been treated only with glyphosate, which increased the pressure for the selection of resistant biotypes. As a consequence, three years after the introduction of GMHT soybean varieties, glyphosate-resistant horseweed (*Conyza canadensis*) has been detected. Knowing that there are alternative herbicides that provide efficient and good weed control, farmers have to add another herbicide to

glyphosate to control the resistant weed species. The adoption of GMHT crops has allowed the use of a single broad spectrum herbicide that may reduce the need for costly herbicide combinations. Glyphosate and glufosinate are generally considered toxicologically more benign, being in particular less toxic to humans and the environment than many of the herbicides they replace. In addition, the adoption of GMHT crops has often facilitated the change to conservation tillage agriculture. Growers using conservation tillage have reduced their tillage operations, thus preventing soil erosion and soil degradation. The results of the UK Farm Scale Evaluations (FSE) showed that weed biomass and numbers of some invertebrate groups were reduced under GMHT management in sugar beet and oilseed rape and increased in maize compared with conventional treatments. These differences were related to the weed management of both conventional and GMHT systems. Highly effective weed control practices, such as those chosen for the GMHT crops in the FSE, lead to low numbers of weed seeds and insects. Fewer insects and decreased weed seed might reduce the numbers of birds that depend on these insects and seeds as a food source. The FSE assumed no other changes in field management than GMHT crops replacing non-GM varieties. Other cropping systems such as conservation tillage are possible, resulting in a greater availability of crop residues and weed seeds and, in consequence, improving food supplies for insects, birds, and small mammals.

When discussing the risks of GM crops, one has to recognize that the real choice for farmers and consumers is not between a GM technology that may have risks and a completely safe alternative. The real choice is between GM crops and current conventional pest and weed management practices, all possibly having positive and negative outcomes. To ensure that a policy is truly precautionary, one should therefore compare the risk of adopting a technology against the risk of not adopting it. We thus believe that both benefits and risks of GM crop systems should be compared with those of current agricultural practices.

Zusammenfassung

Ökologische Auswirkungen gentechnisch veränderter Pflanzen
Erfahrungen aus zehn Jahren experimenteller Feldforschung und kommerziellem Anbau

Seit gentechnisch veränderte Pflanzen (GVP) erstmals im Jahre 1996 kommerziell angebaut wurden hat sich ihre weltweite Anbaufläche kontinuierlich vergrössert und erreichte im Jahre 2005 eine Fläche von 90 Millionen Hektaren. Gentechnisch veränderte Pflanzen werden zurzeit von 8,5 Millionen Landwirten in 21 verschiedenen Ländern angebaut. Neunzig Prozent dieser Landwirte leben in Entwicklungsländern. Nahezu 95% der gesamten Anbaufläche von GVP befindet sich in fünf Ländern, namentlich den USA, Argentinien, Brasilien, Kanada und China. Weltweit werden hauptsächlich vier gentechnisch veränderte (GV) Kulturpflanzen angebaut. Sojabohnen sind flächenmässig die am häufigsten angebaute Kultur, gefolgt von Mais, Baumwolle und Raps. Die Toleranz gegen spezifische Herbizide ist die dominierende Eigenschaft, die mit gentechnischen Verfahren in alle vier Kulturpflanzen eingefügt wurde, während Mais und Baumwolle die beiden einzigen kommerziell angebauten GVP mit einer Resistenz gegen Insekten sind. Es gibt Bedenken, dass der Anbau von GVP zu negativen Auswirkungen auf die Umwelt führen könnte. Die Forschungsanstalt Agroscope Reckenholz-Tänikon ART wurde von der Eidgenössischen Fachkommission für biologische Sicherheit beauftragt, die wissenschaftlichen Erkenntnisse über Umweltauswirkungen von GVP zusammenzufassen, die im Laufe von zehn Jahren experimenteller Feldforschung und kommerziellem Anbau gewonnen wurden. Als Datenbasis dienten wissenschaftliche Fachartikel und Bücher, sowie Berichte aus Ländern mit GVP-Anbau und von internationalen Organisationen. Teilweise standen für einige der untersuchten Fragen, nur wenige Daten aus dem kommerziellen Anbau zur Verfügung. Die meisten Kapitel der Studie enthalten deshalb auch Erkenntnisse, die aus grossflächigen experimentellen Feldversuchen stammen. Die Autoren dieser Studie sind sich bewusst, dass Ergebnisse aus dem grossflächigem Anbau, wie er oftmals in Länder in denen GVP angebaut werden praktiziert wird, mit Vorsicht auf den in der Schweiz typischen kleinräumigen Anbau übertragen werden sollten. Dennoch sind wir der Meinung, dass die Erkenntnisse aus den weltweit vorhandenen wissenschaftlichen Daten und die Erfahrungen aus der Praxis in der Diskussion um mögliche Risiken einer Freisetzung von GVP in der Schweiz berücksichtigt werden sollten.

Die Sicherheit von gentechnisch veränderten Pflanzensorten wird im Vergleich zu konventionell gezüchteten Sorten sorgfältiger untersucht. Zusätzlich zu dem in der klassischen Züchtung durchgeführten Selektionsprozess, ist für die Zulassung einer gentechnisch veränderten Pflanzensorte eine eingehende Risikobewertung möglicher Umweltauswirkungen vorgeschrieben. Seit Beginn des kommerziellen Anbaus von GVP vor zehn Jahren wurden die Risiken für die Umwelt, und speziell für die Biodiversität, daher intensiv untersucht. Folglich existieren heute zahlreiche wissenschaftliche Daten und Erkenntnisse zu den Umweltauswirkungen der im Anbau befindlichen GVP. Weitere Erkenntnisse werden in den nächsten Jahren folgen, da zurzeit noch mehrere Forschungsprogramme in diversen Ländern durchgeführt werden. Die bisher vorliegenden Erkenntnisse liefern keine wissenschaftlich begründeten Hinweise, dass der kommerzielle Anbau

von GVP zu Umweltschäden geführt hat. Dennoch wird bei der Interpretation der vorhandenen wissenschaftlichen Daten eine Reihe von Punkten kontrovers debattiert. Dies liegt daran, dass wissenschaftliche Daten immer mit Unsicherheiten behaftet sind. Zudem sind Voraussagen zu potenziellen langfristigen oder kumulativen Auswirkungen schwierig. Unsicherheiten können zwei Ursachen haben. Zum einen können noch nicht genügend Daten vorhanden sein, um die Konsequenzen abschliessend bewerten zu können (das »Unbekannte«). Andererseits ist es möglich, dass die zu lösenden Fragen mit wissenschaftlichen Methoden nicht beantwortet werden können (das prinzipiell »Nicht-Wissbare«). Die momentan geführte Debatte entstammt nicht primär einem Mangel an Erfahrungen und soliden wissenschaftlichen Daten, sondern eher einer unklaren Interpretation, welche Umweltauswirkung einer GVP als ökologisch relevant zu bewerten sind. Die Interpretation wissenschaftlicher Studien wird oftmals durch das Fehlen einer Vergleichsbasis (der so genannten »baseline«) erschwert, d.h. es fehlen klare Kriterien wie Umweltauswirkungen von GVP im Kontext moderner landwirtschaftlicher Anbaumethoden bewertet werden sollen. Es ist daher nötig, wissenschaftliche Kriterien für die Bewertung von Umweltauswirkungen von GVP zu entwickeln. Mit deren Hilfe können Regulierungsbehörden entscheiden, ob Auswirkungen als relevant zu betrachten sind. In der vorliegenden Studie diskutieren wir die Umweltauswirkungen des kommerziellen Anbaus von GVP im Vergleich zu Auswirkungen, die durch konventionelle landwirtschaftliche Anbausysteme verursacht werden. Unabhängig von der Anwendung von GVP haben moderne landwirtschaftliche Anbausysteme tief greifende Auswirkungen auf alle Umweltressourcen, einschliesslich negativer Auswirkungen auf die Biodiversität. Eine Reihe von Änderungen in der Bewirtschaftung der landwirtschaftlich genutzten Flächen hat im Laufe des letzten Jahrhunderts zu einer Abnahme der Artenvielfalt in der Agrarlandschaft geführt.

Insektenresistente *Bt*-Kulturpflanzen, die Cry-Proteine aus *Bacillus thuringiensis (Bt)* exprimieren, könnten zusätzlich zum Schädling, gegen den das *Bt*-Toxin gerichtet ist, weitere Organismen schädigen. Die Studien, in denen mögliche unerwünschte Auswirkungen auf Nicht-Zielarthropoden in grossflächigen Feldversuchen untersucht wurden, zeigen jedoch nur geringfügige Veränderungen in den Arthropodengemeinschaften die durch das Fehlen des Zielschädlings, als Folge der effizienten Bekämpfung durch die *Bt*-Kulturpflanzen, erklärt werden können. Weder in Labor- noch in Feldstudien konnte eine direkte toxische Wirkung der exprimierten *Bt*-Toxine auf Nützlinge beobachtet werden. Vieles deutet darauf hin, dass die derzeit angebauten *Bt*-Kulturpflanzen im Vergleich zu den meisten heutigen Insektiziden spezifisch nur auf den (oder die) Zielschädling(e) wirken und zu weniger Nebeneffekten auf Nicht-Zielorganismen führen. Während der Anbau von *Bt*-Mais nur zu einer geringen Abnahme der Insektizidanwendungen geführt hat (da konventioneller Mais nur selten mit Insektiziden gegen den Maiszünsler behandelt wird), hat der Anbau von *Bt*-Baumwolle sowohl zu einer substanziellen Reduktion in der Menge als auch in der Anzahl Insektizidanwendungen geführt. Die Abnahme der Insektizidanwendungen in *Bt*-Baumwolle hat, speziell in Entwicklungsländern, nachweislich auch zu gesundheitlichen Vorteilen für die Landwirte geführt.

Bt-Kulturpflanzen könnten, ähnlich den Nicht-Zieleffekten auf oberirdisch lebende Organismen, auch unerwünschte Auswirkungen auf Bodenorganismen haben. *Bt*-Toxine gelangen primär durch Wurzelexudate und mit Pflanzenresten nach der Ernte in den Boden. Sowohl der Abbau als auch die Inaktivierung von *Bt*-Toxinen variiert in Abhängigkeit verschiedener Parameter wie Temperatur und Bodenart. *Bt*-Toxine werden im Boden rasch abgebaut. Da sie an Ton- und Humuspartikel binden können, kann ein geringer Anteil der *Bt*-Toxine (< 2%) nach einer Vegetationsperiode im Boden verbleiben.

Verschiedene Untersuchungen zeigen jedoch, dass nach mehreren Jahren Anbau von *Bt*-Kulturpflanzen keine Akkumulation der Toxine im Boden nachgewiesen werden kann. Die Populationsgrösse und die Zusammensetzung der Gemeinschaft der Bodenorganismen unterliegen natürlicherweise grossen saisonalen Schwankungen und werden zusätzlich durch eine Reihe von Parametern des jeweiligen Anbausystems beeinflusst (Kultur, Sorte und Fruchtfolge). Weder in Labor- noch in Feldstudien sind letale oder subletale Auswirkungen der *Bt*-Toxine auf Bodenorganismen wie Regenwürmer, Springschwänze (Collembolen), Milben, Asseln oder Nematoden festgestellt worden. In der Mikroorganismengemeinschaft des Bodens wurden Unterschiede in der Menge der Organismen und in der Zusammensetzung der Gemeinschaft beobachtet. Die ökologische Relevanz der beobachteten Unterschiede ist weitgehend unklar. Da die in landwirtschaftlichen Anbausystemen natürlich vorkommende Variabilität in den meisten Studien nicht erhoben wurde, ist es generell schwierig zu ermitteln, ob die Unterschiede zwischen *Bt*- und nicht-*Bt*-Kulturpflanzen diese natürliche Variabilität überschreiten. Die im Rahmen einer einzigen Studie erhobenen Daten zur natürlichen Variabilität zeigen, dass die beobachteten Auswirkungen innerhalb dieser Variabilität liegen und dass Unterschiede, die zwischen konventionellen Sorten bestimmt wurden, grösser als die beobachteten Auswirkungen durch *Bt*-Kulturpflanzen sind.

Es ist wissenschaftlich unumstritten, dass Auskreuzung (»gene flow«), d.h. die Übertragung von genetischem Material zwischen GVP und sexuell kompatiblen verwandten Wildarten, stattfinden kann. Experimentelle Studien haben gezeigt, dass GVP spontan mit verwandten Wildarten hybridisieren können, jedoch zu einem ähnlichen Grad wie dies auch bei nicht transgenen Kulturpflanzen zu erwarten ist. In einigen Studien wurde nachgewiesen, dass herbizidtoleranter GV-Raps *(Brassica napus)* mit geringer Häufigkeit unter natürlichen Bedingungen F1-Hybriden mit der Weissen Rübe *(Brassica rapa)* bildet. Es ist unklar, ob die übertragenen Transgene in den Pflanzenpopulationen, die diese Gene aufnehmen, zu ökologisch relevanten Veränderungen führen können. Obwohl die Möglichkeit besteht, dass das Unkrautpotenzial durch Auskreuzung erhöht wird, ist es eher unwahrscheinlich, dass herbizidtolerante Unkräuter stärkere agronomische Probleme als normale Unkräuter verursachen würden. Die Landwirte können beim Anbau einer Kultur in der Regel zwischen verschiedenen Herbiziden wählen und haben zudem innerhalb einer Fruchtfolge verschiedene Optionen der Unkrautbekämpfung.

In natürlichen Habitaten wurde bisher keine langfristige Introgression von gentechnisch veränderten Sequenzen in Populationen wilder Pflanzenarten beobachtet, die zum Aussterben einer wilden Pflanzenart geführt hätte. Da Herbizidtoleranz-Gene in natürlichen Habitaten selektionsneutral sind, ist es unwahrscheinlich, dass herbizidtolerante Pflanzen in natürlichen Habitaten einen Selektionsvorteil besitzen. In Fällen wo Schädlinge zur Kontrolle natürlicher Pflanzenpopulationen beitragen, könnten Gene zur Erzeugung einer Insektenresistenz hingegen die Fitness der Pflanze erhöhen.

In der Diskussion um mögliche Umweltrisiken von GVP wurden schon früh Bedenken geäussert, dass sich diese Pflanzen in natürliche Habitate ausbreiten könnten. Moderne Kultursorten besitzen jedoch nur ein geringes Verwilderungspotenzial und verbleiben daher in der Regel innerhalb der Kulturflächen. Die Erfahrungen, die in den letzten Jahren im Westen Kanadas gemacht wurden, geben keine Hinweise, dass der intensive Anbau von herbizidtolerantem Raps zu einer grossräumigen Verbreitung von herbizidtolerantem Ausfallraps geführt hat. Obwohl in einer Studie dreifach-herbizidresistenter und in einer zweiten Studie zweifach-herbizidresistenter Ausfallraps nachgewiesen werden konnte, legt der allgemeine Mangel an Berichten über mehrfach-herbizidresis-

tenten Ausfallraps den Schluss nahe, dass dieser durch chemische und mechanische Bekämpfungsstrategien kontrolliert werden kann und deshalb für die meisten Landwirte kein agronomisches Problem darstellt. Zudem gibt es zurzeit keine Hinweise, dass herbizidtoleranter GV Raps verwildert ist und sich in natürlichen Habitaten verbreitet hat.

Der Einfluss von GVP auf das Management von Unkräutern und Schädlingen und die daraus möglicherweise entstehenden ökologischen Konsequenzen sind schwierig zu ermitteln. Mögliche Auswirkungen werden von diversen interagierenden Faktoren beeinflusst und treten oftmals erst nach einem längeren Zeitraum auf. Bereits vor Einführung von herbizidtoleranten GVP haben zahlreiche Unkrautarten Resistenzen gegen eine Reihe von Herbiziden entwickelt. Die Erkenntnisse aus Gegenden mit grossflächigem Anbau von herbizidtoleranten GVP bestätigen, dass die Entwicklung von Herbizidresistenzen in Unkräutern nicht primär eine Frage der Gentechnik, sondern des Anbau- und Herbizidmanagements ist. Trotz des grossflächigen Anbaus von herbizidtolerantem GV Raps in Kanada wurden bisher noch keine Unkräuter beobachtet, die gegenüber den Herbiziden Glyphosat und Glufosinat resistent sind. Andererseits hatte die Einführung von herbizidtoleranten GV Sojasorten in den USA zur Folge, dass in Monokulturen viele Felder nur noch mit Glyphosat behandelt wurden. Dies erhöhte den Selektionsdruck für resistente Biotypen, so dass Glyphosat-Resistenzen im Kanadischen Berufkraut *(Conyza canadensis)* bereits drei Jahre nach Einführung der herbizidtoleranten GV Sojasorten nachgewiesen wurden. Zur Bekämpfung herbizidresistenter Unkräuter müssen die Landwirte neben Glyphosat ein zusätzliches Herbizid einsetzen. Es existieren jedoch andere Herbizide, die eine gute Unkrautbekämpfung und Flexibilität in der Anwendung gewährleisten. Herbizidtolerante GVP erlauben den Einsatz eines einzigen Breitbandherbizids, wodurch aufwändige Herbizidkombinationen verringert werden können. Glyphosat und Glufosinat werden im Vergleich zu vielen eingesetzten Herbiziden als weniger toxisch für die menschliche Gesundheit und die Umwelt beurteilt. Die Einführung von herbizidtoleranten GVP hat vielerorts den Wechsel zu einer konservierenden pfluglosen Bodenbearbeitung gefördert. Auf diese Weise können Landwirte Bodenerosion effizient vermeiden. In den englischen Farm Scale Evaluations (FSE) wurde beobachtet, dass die Unkrautbiomasse und die Artenzahlen gewisser Insektengruppen beim Anbau von herbizidtolerantem GV Raps und von GV Zuckerrüben im Vergleich zur konventionellen Unkrautbekämpfung reduziert waren. Beim Anbau von herbizidtolerantem GV Mais hingegen waren die Unkrautbiomasse sowie die Artenzahlen gewisser Insektengruppen erhöht. Die beobachteten Unterschiede können durch die unterschiedliche Unkrautbekämpfung in den beiden Anbausystemen mit und ohne Gentechnik erklärt werden. Eine sehr effektive Unkrautbekämpfungs-Strategie, wie sie für die herbizidtoleranten GVP in den FSE gewählt wurde, führt zu einer geringeren Anzahl von Unkräutern respektive Unkrautsamen und Insekten. Weniger Unkrautsamen und Insekten könnten entsprechend das Futterangebot für Vögel reduzieren, die auf diese Nahrungsquellen angewiesen sind. Die FSE gingen von der Annahme aus, dass die nicht-transgenen Sorten einzig durch herbizidtolerante GVP ersetzt würden und sonst keine weiteren Änderungen in der Anbautechnik stattfinden. Ob diese Annahme in der Praxis zutrifft ist schwer zu beurteilen, da der Anbau von herbizidtoleranten GVP auch andere Anbausysteme ermöglicht, so beispielsweise eine konservierende pfluglose Bodenbearbeitung. Diese hat eine grössere Verfügbarkeit von Pflanzenresten und Unkrautsamen zur Folge, was wiederum das Nahrungsangebot für Insekten, Vögel und Kleinsäuger verbessern kann.

In der Diskussion um Risiken von GVP sollte berücksichtigt werden, dass Landwirte und Konsumenten nicht zwischen der Pflanzenbiotechnologie mit ihren möglichen Risiken und einer vollkommen sicheren Alternative wählen können. In Wirklichkeit besteht

die Wahl zwischen Anbausystemen mit GVP oder Anbausystemen mit den heute üblichen Schädlings- und Unkrautbekämpfungsstrategien, die beide sowohl positive wie negative Folgen haben können. Wir sind deshalb der Meinung, dass bei Entscheidungen zur Anwendung der Pflanzenbiotechnologie Risiken und Nutzen aller Anbausysteme miteinander verglichen werden sollten.

Résumé

Impacts écologiques des cultures génétiquement modifiées
Expériences de dix ans de recherche expérimentale en champ et de culture commerciale

Depuis que les cultures génétiquement modifiées ont été utilisées pour la première fois à des fins commerciales en 1996, leur superficie mondiale n'a cessé d'augmenter pour atteindre 90 millions d'hectares en 2005. Les cultures génétiquement modifiées sont actuellement gérées par 8,5 millions de cultivateurs répartis dans 21 pays; 90% de ces personnes vivent dans des pays en développement. Près de 95% de la superficie totale de ces cultures sont situés dans cinq pays (Etats-Unis, Argentine, Brésil, Canada et Chine). Le soja est la plante transgénique la plus largement cultivée au monde. Viennent ensuite le maïs, le coton et le colza. La tolérance aux herbicides est la principale propriété qui a été attribuée à ces quatre plantes par des méthodes de modification génétique. Quant aux plantes transgéniques résistantes aux insectes, seuls le maïs et le coton sont actuellement disponibles sur le marché. La culture commerciale des plantes transgéniques a fait craindre que ces pratiques puissent avoir des effets néfastes sur l'environnement. La Commission fédérale d'experts pour la sécurité biologique a donc chargé la Station de recherche Agroscope Reckenholz-Tänikon ART de récapituler les connaissances scientifiques sur les effets environnementaux des cultures génétiquement modifiées en examinant le savoir acquis pendant dix ans de recherche expérimentale en champ et de culture commerciale. Les sources d'information proviennent de revues et de livres scientifiques spécialisés, de rapports issus de pays pratiquant ce type de culture et de rapports d'organisations internationales. Pour certaines questions concernant la culture commerciale, les données disponibles étaient très limitées. En conséquence, la plupart des chapitres de cette étude contiennent également des données scientifiques collectées lors de recherches expérimentales en champ à grande échelle. Les auteurs reconnaissent que les résultats obtenus pour des systèmes de culture conduits à grande échelle, très répandus dans les pays où se trouvent les cultures génétiquement modifiées, sont à reporter avec une certaine prudence aux systèmes de culture conduits à une échelle plus fine, comme en Suisse. Ils pensent néanmoins que les données scientifiques de portée mondiale devraient être prises en compte lors de décisions futures et dans les débats sur les risques potentiels d'une dissémination expérimentale de plantes transgéniques en Suisse.

La sécurité des cultures génétiquement modifiées est généralement évaluée plus minutieusement que celle des plantes issues de croisements conventionnels. Car en plus du processus de sélection réalisé pour les cultures classiques, une appréciation détaillée des risques d'effets indésirables sur l'environnement est exigée par la loi pour obtenir l'autorisation de commercialiser toute variété de plante transgénique. Depuis le début de la culture commerciale de ces plantes il y a dix ans, les risques pour l'environnement, et notamment pour la biodiversité, ont été suivis de près. Il existe donc aujourd'hui une moisson de données scientifiques sur les effets environnementaux des cultures génétiquement modifiées et actuellement commercialisées. Cet acquis va encore s'élargir ces prochaines années, car plusieurs programmes de recherches sont encore en cours dans divers pays. Les données disponibles aujourd'hui n'apportent aucune preuve scientifique

que les cultures commerciales de plantes génétiquement modifiées aient causé des dommages à l'environnement. Néanmoins, l'interprétation des données scientifiques fait l'objet de controverses. Jusqu'à un certain point, cela est dû au fait que ces données sont toujours caractérisées par des incertitudes et qu'il est difficile de prévoir les effets potentiels sur le long terme ou les effets cumulés. Les incertitudes sont liées à l'insuffisance des bases de données permettant d'évaluer les conséquences (« l'inconnu ») ainsi qu'à l'impossibilité de répondre aux questions posées en s'aidant de méthodes scientifiques (« l'inconnaissable »). Bien que l'on puisse argumenter sur le manque d'expérience et de données scientifiques fondées, les débats ne portent pas principalement sur cette question, mais plutôt sur l'ambiguïté de l'interprétation des éléments à considérer dans l'appréciation des effets environnementaux d'une culture génétiquement modifiée. Dès lors, l'interprétation des résultats des études est souvent rendue difficile par l'absence d'une base de comparaison de ces effets dans le contexte des systèmes de culture modernes. Il est donc nécessaire de développer des critères d'appréciation scientifique qui aideront les autorités à juger de l'importance de ces effets. La présente étude fait état des effets de la culture des plantes transgéniques sur l'environnement en comparaison avec les effets causés par les pratiques agricoles des systèmes de culture modernes. Indépendamment de l'utilisation de plantes transgéniques, les systèmes de culture modernes ont des incidences notables sur toutes les ressources environnementales, ainsi que des conséquences négatives sur la biodiversité. Nombre de changements survenus dans la gestion des surfaces agricoles au cours du siècle dernier ont entraîné un appauvrissement de la diversité spécifique dans l'agro-écosystème.

Il est concevable que les plantes transgéniques résistantes aux insectes et qui produisent les protéines Cry issues du *Bacillus thuringiensis (Bt)* puissent aussi affecter d'autres organismes que les ravageurs ciblés par la toxine *Bt*. Cependant, les études à grande échelle concernant les éventuels effets indésirables sur les arthropodes non ciblés montrent que la communauté d'arthropodes n'a subi que de minimes changements. Ces résultats peuvent être expliqués par l'absence du ravageur ciblé suite au contrôle efficace par les plantes *Bt*. Les examens en laboratoire et en champ n'ont indiqué aucun effet néfaste produit par la toxicité directe de la toxine *Bt* sur les insectes auxiliaires non ciblés. Il a été prouvé que les plantes *Bt* cultivées actuellement ont un mode d'action plus spécifique et produisent moins d'effets secondaires sur les organismes non ciblés que la plupart des insecticides actuels. Tandis que la culture du maïs *Bt* ne s'est accompagnée que de modestes réductions d'application d'insecticides (car les surfaces de maïs conventionnel traitées contre la pyrale du maïs sont peu étendues), la culture commerciale du coton *Bt* a permis de réduire considérablement les quantités et le nombre de ces applications. En plus des avantages directs pour l'environnement, il est prouvé que la diminution de ces traitements dans les cultures de coton *Bt* a été bénéfique pour la santé des cultivateurs en particulier dans les pays en développement.

Au même titre que ce qui a été observé pour les organismes non ciblés cités précédemment, la question a été de savoir si les plantes *Bt* pouvaient avoir des effets indésirables sur les organismes du sol. Les toxines *Bt* pénètrent dans le sol en passant principalement par les excrétions des racines et par les résidus de récolte. La dégradation et l'inactivation de la toxine *Bt* varient en fonction de divers paramètres, comme la température et le type de sol. La dégradation initiale de la toxine est rapide; il est cependant possible qu'un faible pourcentage (< 2%) subsiste dans l'écosystème du sol après la période de cultivation, car les toxines *Bt* peuvent se fixer sur des particules d'argile et d'acide humique. Néanmoins, aucune accumulation de toxines n'a été observée dans le sol après plusieurs années de culture de plantes *Bt*. La taille des populations et la structure des

communautés des organismes du sol sont soumises à de fortes variations naturelles dues aux saisons et sont influencées par divers paramètres liés au système de culture (type de sol, âge de la plante, culture, cultivar et rotation des cultures). Les études réalisées tant en laboratoire qu'en champ n'ont permis de déceler aucun effet létal ou sous-létal de la toxine *Bt* sur les organismes du sol, tels que les lombrics, les collemboles, les acariens, les isopodes ou les nématodes. Quelques différences s'inscrivent dans le nombre total et la structure des communautés de microorganismes. La signifiance écologique de ces différences est incertaine. Etant donné que la variabilité naturelle propre aux systèmes de culture agricole n'a pu être relevée dans la majorité des études, il est généralement diffi- cile de dire si les différences entre les plantes *Bt* et les plantes non *Bt* excèdent cette vari- abilité. La seule étude considérant ce facteur tend à conclure que les effets constatés restent dans la marge de cette variabilité et que les différences entre les cultivars conven- tionnels vont au-delà des influences exercées par les plantes *Bt*.

Il est scientifiquement incontesté qu'un flux de gènes, c'est-à-dire un transfert de matériel génétique, peut intervenir entre les plantes transgéniques et les espèces sau- vages apparentées sexuellement compatibles. Des recherches expérimentales ont montré que les plantes transgéniques sont capables de s'hybrider spontanément avec des espèces sauvages apparentées, mais dans une mesure semblable à ce qui peut se pro- duire parmi les plantes non modifiées génétiquement. Quelques études ont démontré que le colza *(Brassica napus)* transgénique tolérant aux herbicides peut former des hybrides F1 avec le navet *(Brassica rapa)* dans des conditions naturelles. On ne sait pas encore clairement si les séquences génétiquement modifiées peuvent causer des change- ments écologiques notables dans les populations de plantes qui reçoivent ces gènes. Il est peu probable que le flux de gènes puisse augmenter le potentiel de développement des populations de mauvaises herbes. Il n'y a donc pas lieu de supposer que les mau- vaises herbes tolérantes aux herbicides puissent poser des problèmes agricoles plus importants que les mauvaises herbes conventionnelles. Les cultivateurs peuvent en général choisir entre plusieurs herbicides pour une culture donnée et ils ont en outre plusieurs options pour lutter contre les mauvaises herbes dans une rotation culturale.

Dans les habitats naturels, les chercheurs n'ont constaté aucune introgression à long terme de séquences génétiquement modifiées susceptibles d'entraîner l'extinction d'une espèce de plante sauvage au sein de ces populations. Etant donné que les gènes produi- sant une résistance aux herbicides sont sélectivement neutres dans les habitats naturels, il est improbable que les plantes tolérantes aux herbicides possèdent un avantage sélectif dans ces lieux. En revanche, les gènes conférant la résistance aux insectes pourraient favoriser la fitness si les ravageurs contribuent au contrôle des populations naturelles de plantes.

Lors des débats sur les risques environnementaux liés aux cultures génétiquement modifiées, l'éventualité que des plantes transgéniques se répandent dans les habitats naturels a très tôt préoccupé les esprits. Or, on sait aujourd'hui que les variétés de plantes issues de cultures modernes restent généralement au sein des parcelles cultivées. D'après l'expérience faite ces dernières années dans l'Ouest du Canada, il n'existe aucun indice laissant supposer que la culture extensive de colza tolérant aux herbicides aurait conduit à une dissémination à grande échelle de ce type de plante. Deux études mentionnent la présence de repousses de colza dotées d'une double, voire d'une triple résistance aux herbicides. Toutefois, le manque général de rapports concernant la résistance multiple de ces repousses nous prête à conclure que les stratégies de gestion chimique et mécanique permettent de maîtriser cette situation qui n'est donc pas perçue comme un problème par la majorité des cultivateurs. Par ailleurs, il n'existe actuellement aucune évidence que

du colza transgénique résistant aux herbicides soit devenu une espèce sauvage et se soit disséminé dans des habitats naturels.

L'influence des cultures génétiquement modifiées sur la gestion des mauvaises herbes et des ravageurs ainsi que les conséquences écologiques qui peuvent s'ensuivre sont généralement difficiles à estimer. Ces impacts sont en effet souvent influencés par de nombreux facteurs en interactions et ne se manifestent parfois qu'après une longue période. Bon nombre d'espèces de mauvaises herbes ont développé une résistance à beaucoup d'herbicides bien avant l'introduction de plantes transgéniques résistantes aux herbicides. L'expérience acquise en la matière dans des régions pratiquant la culture à grande échelle confirme que le développement de résistances aux herbicides chez les mauvaises herbes n'est pas lié aux modifications génétiques elles-mêmes mais davantage aux pratiques culturales et de gestion des mauvaises herbes. Au Canada, malgré la culture intensive de colza transgénique tolérant aux herbicides, aucune espèce de mauvaise herbe présentant une résistance au glyphosate et au glufosinate n'a été décelée jusqu'à ce jour. Par contre, dans des cultures de soja transgénique tolérant aux herbicides qui ont été conduites de façon ininterrompue aux Etats-Unis, la pression de sélection de biotypes résistants a été accrue du fait de traitements exclusifs aux glyphosates. Cela a conduit à l'apparition de vergerette du Canada *(Conyza canadensis)* résistante au glyphosate trois ans après l'introduction de cultivars de soja transgénique. Dans leur gestion des mauvaises herbes tolérantes aux herbicides, les cultivateurs doivent ajouter un autre herbicide au glyphosate. Il existe des produits de remplacement très efficaces qui garantissent un bon résultat et permettent une application souple. L'adoption de cultures génétiquement modifiées et tolérantes aux herbicides permet en outre d'utiliser un seul herbicide à large spectre, ce qui réduit les coûts par rapport aux traitements combinant plusieurs herbicides. On considère généralement que le glyphosate et le glufosinate ont des effets moins toxiques pour la santé humaine et pour l'environnement que les herbicides qu'ils remplacent. Enfin, l'adoption de cultures génétiquement modifiées et tolérantes aux herbicides a souvent facilité le passage à un travail de conservation du sol sans labour. Les cultivateurs ayant adopté cette pratique ont simplifié ce travail tout en prévenant l'érosion et la dégradation du sol. Dans l'étude « Farm Scale Evaluations » (FSE) réalisée en Angleterre, les chercheurs ont comparé la gestion des mauvaises herbes pratiquée dans les cultures conventionnelles avec celle qui est adoptée dans les cultures tolérantes aux herbicides. Les résultats montrent que la biomasse en mauvaises herbes et le nombre de quelques groupes d'invertébrés sont moins élevés dans les cultures de colza et de betterave à sucre tolérantes aux herbicides que dans les cultures conventionnelles, alors qu'ils sont supérieurs dans celles de maïs du même type. Une pratique de gestion des mauvaises herbes très efficace, comme celle qui a été choisie pour les cultures génétiquement modifiées dans l'étude FSE, conduit à une diminution du nombre d'insectes et de graines de mauvaises herbes. Cela peut toutefois entraîner une réduction du nombre d'oiseaux dont la vie dépend de ces sources de nourriture. La FSE admet que le seul changement intervenant dans la pratique culturale serait le remplacement des espèces non transgéniques par des espèces transgéniques tolérantes aux herbicides. Il est également possible d'adopter d'autres pratiques culturales, comme le travail de conservation du sol sans labour. Ces systèmes favorisent la présence de résidus de récolte et de graines de mauvaises herbes, ce qui améliore aussi la quantité de nourriture offerte aux insectes, oiseaux et petits mammifères.

Dans les débats sur les risques liés aux cultures génétiquement modifiées, force est de reconnaître que les cultivateurs et les consommateurs ne peuvent véritablement choisir entre le génie génétique des plantes, qui peut comporter des risques, et une alternative

entièrement sûre. En réalité, il s'agit de choisir entre les systèmes de cultures génétique-ment modifiées et les actuelles pratiques courantes de gestion des ravageurs et des mauvaises herbes. Les deux peuvent avoir des conséquences tant négatives que posi-tives. Pour s'assurer qu'une réglementation répond vraiment au principe de précaution, il faudrait peser le risque qui est pris en adoptant une technologie ou en y renonçant. Nous estimons qu'il serait utile de comparer les avantages et les risques des systèmes de cultures génétiquement modifiées avec ceux des pratiques culturales actuelles.
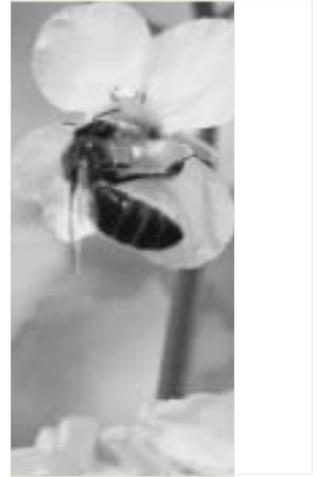
1 Introduction

1.1 Background

Since genetically modified (GM) crops were first commercialized in 1996, farmers have consistently increased their plantings of GM crops by 10% or more each year worldwide. It is generally expected that commercial cultivation of GM crops will further increase over the coming years. In contrast to this worldwide trend, the adoption of GM crops in Europe was much less intense. Apart from Spain, where commercial GM crop cultivation started in 1998, no other European country was commercially growing GM crops up to 2004. This situation is probably going to change, since the European Union (EU) entered the first GM maize varieties expressing insecticidal proteins from *Bacillus thuringiensis (Bt)* into the Common EU Catalogue of Varieties in September 2004. It is generally expected that *Bt*-maize will also be commercially grown in other EU countries. Several countries such as France, Germany, Portugal and the Czech Republic have started growing *Bt*-maize in 2005. Compared to Spain where approximately 12% of the total maize area grown in 2004 (representing 58'000 ha) was planted with *Bt*-maize, the acreage in these countries is, however, very limited and accounts for less than 1'000 ha each.

The commercial cultivation of GM crops in Switzerland, in contrast, seems to be rather unlikely in the foreseeable future. There is a strong opposition against the use of GM technology in Swiss agriculture, which resulted in the approval of a five year moratorium for commercial cultivation of GM crops by Swiss voters in November 2005. It is, however, probable that there will be further field trials with GM crops in the near future, especially within the National Research Programmes "Risks and benefits of genetically modified organisms", which has recently been approved by the Swiss National Science Foundation and which is supposed to start in 2007.

The Swiss Gene Technology Law (GTL) stipulates that genetically modified organisms shall be handled in such a way that they cannot endanger humans, animals, and the environment. Furthermore they shall not impair biological diversity or the sustainable use thereof. According to the GTL, the Swiss Expert Committee for Biosafety (SECB) advises Swiss Authorities on the protection of people and the environment in the area of bio-

technology. Within the approval process for field trials and commercial cultivation of GM crops, the SECB issues recommendations on safety issues of GM crops. Where necessary, the SECB can initiate external expertise and research.

1.2 Objectives of the study

Agroscope Reckenholz-Tänikon Research Station ART was commissioned by the SECB to review the available scientific data on ecological effects of GM crops, which originates from ten years of large-scale experimental field trials and commercial cultivation. Furthermore, the environmental effects and risks of GM crop cultivation will be compared with effects caused by cultivation practices of modern agricultural systems.

1.3 Content of the study

The study is concentrating on the currently commercially available GM crops that could be relevant for Swiss agriculture such as maize, oilseed rape and soybean. Where helpful, experiences gained with other crops like *Bt*-cotton are considered. The study is focusing on the two main GM traits that are currently commercialized, "Herbicide tolerance (HT)" and "Insect resistance (IR)". GM crops with minor worldwide acreage (such as virus-resistant papaya and squash) are not considered. Potential effects of GM crops are limited to the environment and the six following topics are considered:

(1) Effects of GM crops on non-target organisms
(2) Effects of GM crops on soil ecosystems
(3) Gene flow from GM crops to wild relatives
(4) Invasiveness of GM crops into natural habitats
(5) Impacts of GM crops on pest and weed management
(6) Ecological benefits of GM crop cultivation

In accordance with the mandate given by the SECB, some potential environmental concerns related to the use of GM crops were not specifically considered. These include the persistence and fate of transgenic DNA in the environment, horizontal gene transfer (such as the transfer of genetic material from plants to bacteria), and insect resistance management (i.e. the strategies undertaken to prevent resistance of the target pest(s) towards the toxins expressed in insect resistant GM crops). Economic issues as well as effects of GM crops on human and animal health are only discussed where necessary.

1.4 Transferability of worldwide field data to Swiss agriculture

The authors recognize that one should be cautious when transferring field data on environmental impacts of GM crops coming from countries with commercial cultivation to the small-scale agricultural systems of Switzerland. These GM crops are often grown in large scale agricultural systems and under "conventional" agricultural practices, whereas in Switzerland more than 95% of the arable land is managed according to integrated production (IP) guidelines. Due to various differing factors a comparison of agricultural systems among countries can thus be difficult. Nevertheless, some of the scientific data reviewed in this study has been obtained under experimental conditions, which we believe are transferable to the Swiss agricultural context. We thus suggest that the scientific knowledge reviewed in this study should be taken into account for future decision making when discussing field releases of GM crops in Switzerland.

2 Methodology of the study

Several questions were addressed during the review of scientific data on ecological effects of GM crops: (1) what is the current knowledge based on peer-reviewed literature and other sources of information, (2) is the knowledge debated controversially by scientists, or is there general scientific agreement, (3) are there gaps in our knowledge or scientific uncertainties and are these important and (4) if there is recognized scientific uncertainty, which options exist?

The sources of information mainly consisted of publications from peer-reviewed scientific journals and from scientific books. Other sources of information included selected reports from countries with extensive GM crop cultivation, such as the USA, Canada, as well as reports from international organizations, which could be readily accessed via the internet. The intention to gain access to unpublished reports submitted to regulatory agencies by the industry by directly contacting several authorities in GM crop cultivating countries did not result in much additional information. The lack of industry data from commercial cultivation is due to the fact that none of the main GM crop growing countries require legally binding post-market monitoring (PMM) activities on potential environmental impacts of GM crops (Jaffe 2004). The regulatory frameworks of these countries recognize that products that have received regulatory approval are judged to be substantially equivalent, and do not present a greater risk than comparable products with a history of safe use (see chapter 4.2.1). However, in some countries such as the United States and Canada, every company putting GM seeds on the market has to report to the regulatory authorities any information on potential adverse effects that has occurred during commercial cultivation of one of their products. Unfortunately, the companies keep the data of their post-market monitoring activities, or they remain within the regulatory authorities since they are considered to be confidential business information. Nevertheless, public sector research has provided substantial scientific data, which is deriving from large-scale experimental field research performed under commercial cultivation conditions.

In Spain, apart from the data that has been published in the scientific literature, not much information obtained from large-scale cultivation was available. This is probably due to the fact that the GM crop varieties (e.g. *Bt*-maize MON810 and Bt11) that are presently grown in the EU have been approved according to the former directive 90/220/EC, which did not mandate post-market monitoring as the new Directive 2001/18/EC.

The published scientific data reviewed within this study is thus often not deriving from commercial cultivation in the proper sense of the term, but more from large-scale experimental field studies, which have been conducted under realistic agricultural conditions.

3 The worldwide cultivation of GM crops

3.1 Global status by country, crop and trait

In 2005 the estimated global area of GM crops was 90 million hectares with an annual growth rate of 11% compared to 2004 (James 2005). GM crops are currently grown by 8.5 million farmers in 21 countries, where 90% of the farmers using the GM technology originate from developing countries. Five countries (USA, Argentina, Brazil, Canada, and China) are growing nearly 95% of these crops with the United States accounting for more than 55% of the worldwide GM crop cultivation (Fig. 1). Apart from the five principal countries, there are some countries with increasing GM crop cultivation. Paraguay, for example, reported the cultivation of 1.8 Mio hectares of GM soybean. India had, based on the annual percentage growth, the highest year-to-year growth with an increase of the *Bt*-cotton area from 0.1 Mio ha in 2003 to 1.3 Mio ha in 2005 counting for almost 15% of its total cotton area planted (James 2004, 2005). Additionally, there are various countries, which commercially grow GM crops on a smaller scale such as South Africa, Uruguay, Australia, Romania, Mexico, Spain and the Philippines.

Figure 1: Estimated global area of genetically modified crops by country (James 2005).
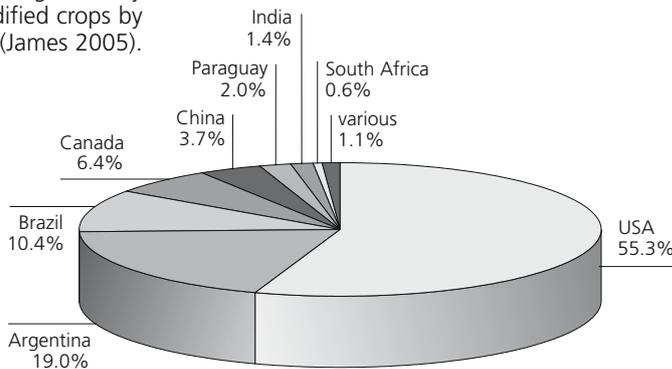
There are four main GM crops that are grown worldwide with soybean occupying most of the global biotech area (60%), followed by maize (24%), cotton (11%) and oilseed rape (5%). Herbicide tolerance is the dominant GM trait that is deployed in soybean, maize, oilseed rape and cotton and grown on 71% of the global biotech crop area. Insect resistant *Bt*-crops (maize and cotton) account for 18% of the global biotech crop area while stacked genes for herbicide tolerance and insect resistance (both in cotton and in maize) occupy 11%.
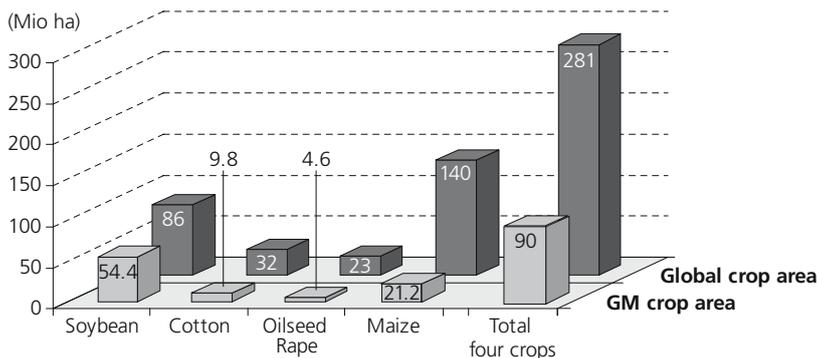
Figure 2: Adoption rates for the four principal GM crops expressed as the percentage of GM crop area of the respective global crop areas (based on James 2005).

The adoption rates of the four major GM crops compared to their respective global crop areas are relatively high, considering that 56% of the global soybean acreage is planted with GM crops. The corresponding figures are 28% for cotton, 19% for oilseed rape and 14% for maize (Fig. 2). The sum of the GM crop area of the four principal GM crops corresponds to 32% of their aggregated global crop area. The adoption rates per crop in specific countries are even higher than the average numbers (Tab. 1). In Argentina and in Uruguay for example, GM soybean were grown on nearly 99% of the total soybean area in 2005, while in the United States and in Romania the number was 87% and 65%, respectively. In Canada, GM oilseed rape is grown on 75% of the total oilseed rape area while in South Africa GM cotton accounts for approximately 85% of the total cotton cultivation.

Table 1: Adoption rates of the four principal GM crops in 2005 (expressed as the percentage of GM crop area of the respective total crop area) (James 2005, TransGen 2006).

Country	GM crop area as percentage of total crop area			
	Soybean	Maize	Cotton	Oilseed rape
USA	87	55	79	76
Argentina	98	55[2]	20 – 25	--
Canada	58[1]	50[1]	--	75
Brazil	39	--	--	--
China	--	--	60	--
Paraguay	60[2]	--	--	--
India	--	--	15	--
South Africa	47[2]	12[2]	85[2]	--
Uruguay	99	?	--	--
Australia	--	--	81[2]	--
Romania	65	--	--	--
Mexico	26[1]	--	36[1]	--
Spain	--	12	--	--

[1] Crop area in 2003, [2] Crop area in 2004
-- No cultivation of this particular GM crop

3.2 Possible future GM crops

As described above, only a limited number of GM crops are currently available as commercial products. Apart from the four principal GM crops, this includes a few other crops (alfalfa, papaya, squash, rice) and some other traits (virus resistance and male sterility/fertility restoration) (Nickson 2005, AGBIOS 2006). This portfolio has a narrow diversity when compared to the number of plants that can be transformed using genetic engineering techniques. Despite successful technological advances, several reasons may be responsible for preventing, delaying or withdrawing GM crops from the market. Apart from marginal consumer acceptance of transgenic crops (such as wheat), vegetables or fruits for direct human consumption potentially affecting their marketability, one of the main reasons for the current focus of industry on four major crops is purely economical and related to the disproportionately high financial hurdle associated with the process of placing GM crops on the market. Due to the many regulatory and legislative requirements for the approval of GM crops, small and medium enterprises cannot afford the cost involved in developing novel GM crops. The situation is further complicated as the majority of transgenic research today is carried out by the public sector, which cannot afford entering these regulatory processes, and which has not the primary objective to commercialize these products. The cost of maintaining just one crop breeding program is estimated to be more than 1.8 mio US$ per year, meaning that these programs are monopolized by five or six international agrochemical companies (van Montagu 2005).

3.2.1 Expansion of input traits

Research efforts of the first generation of GM crops concentrated on so-called input traits, i.e. on traits such as herbicide tolerance and insect resistance that are associated with reducing and substituting certain inputs in agricultural production systems. In the immediate future, expanded use of *Bt*- and GMHT crops will be one of the major focus areas for seed companies and technology providers (Nickson 2005). Both traditional

breeding and modern biotechnology are being used to combine different traits (stacking) such as *Bt* with HT or different variants of the *Bt* gene family. Stacking of *Bt* and HT is an attractive commercial opportunity for the industry because the technology is already established and stacked varieties can be sold at a higher price to growers. Similarly, a stacked GM crop variety that combines two *Bt*-traits Cry3Bb and Cry1Ab to control two different pests [Corn rootworms (*Diabrotica spp.)* and Corn borers (e.g. *Ostrinia nubilalis*)] is close to being marketed in the United States. Another strategy used in the development of insect-resistant GM crops is the combination of two genes with different modes of action (pyramiding) to allow for a more effective control of the same pest complex, as for example in Bollgard II *Bt*-cotton containing two *Bt*-genes (*cry1Ac* and *cry 2Ab*). The use of two different modes of action further provides better insect resistance management in the target pest (Bates *et al.* 2005). A number of other insect resistance traits are under investigation for potential market release including different non-*cry*-gene based insect resistance strategies. These proteins usually have a broader range of insecticidal activity and include Vip3A, Toxin A, avidin, biotin, protease inhibitors, lectins and others (Babu *et al.* 2003, Moar 2003, O'Callaghan *et al.* 2005, Ferry *et al.* 2006).

There are attempts to confer disease resistance to potatoes and to wheat using genetic engineering. In 2006, field trials with GM potatoes resistant to potato late blight (*Phytophthora infestans*) are performed in several countries of the EU, while GM wheat resistant to the fungus Fusarium is currently tested in field trials in Canada. The aim of these field trials is to test if the resistance conferred by genetic engineering is successful in controlling the respective fungus. If the concepts prove to be practicable the genes might be transferred to commercial varieties, which, however, will probably not be available in the near future.

3.2.2 Tolerances to abiotic stresses

Genetic engineering is commonly offered as a hope to improve crop production efficiency by enhancing crop tolerance to various abiotic stresses such as drought, salt and water (Wang *et al.* 2003, Yamaguchi & Blumwald 2005). A basic understanding of the influencing and controlling factors, however, leads to the rather pessimistic view that transformations of a few, or even of a complex of genes will not directly result in major yield increases (Sinclair *et al.* 2004). Forty years of biochemical and physiological research illustrate the great difficulty in translating research at the basic level into improving negative consequences of abiotic stresses on plants. While research into molecular mechanisms of stress responses has started to bear fruits, and genetic modification of stress tolerance has shown promising results that may ultimately apply to agricultural important plants (Wang *et al.* 2003), only few stress-resistant transgenic crops have been evaluated in field trials under real stress conditions (Dunwell 2000). In addition, most of the stress-tolerant GM plants generated to date are non-agronomic plants, making an overall conclusion on the potential commercialization of stress-tolerant GM crops almost impossible.

3.2.3 Output traits

In addition to the development of input traits, the emphasis in research was also early directed towards the development of so-called output traits improving the characteristics or the quality of the harvested product. Examples for this second and third generation of GM crops include attempts to elevate the levels of essential nutrients in food and feed (such as golden rice, Al-Babili & Beyer 2005), to reduce levels of naturally occurring antinutrients and allergens, as well as modifying plant composition enabling more efficient processing into a final product (so-called plant-made industrial) (Nickson 2005).

Although an example for a plant-made industrial, a GM potato with modified starch content, is currently undergoing approval for commercial cultivation in the EU, most GM functional foods and plant-made industrials are still far from market release (Sauter 2005). Judging field trial notifications in the EU, it can be concluded that the development of most output traits is generally still in the early stages of research (Lheureux & Menrad 2004).

There are attempts to use biotechnology to generate plants that produce specific plant-derived pharmaceuticals (PDP), products that are traditionally synthesized using recombinant microbes or transformed mammalian cells (Gomord *et al.* 2005, Ma *et al.* 2005). Considering the large and increasing importance of these biopharmaceuticals and judging their foreseeable economic potential, it is probable that the opportunity to produce these substances in GM plants will increase (Sauter 2005). There are still no commercialized pharmaceutical products derived from plant biotechnology, but several PDPs for human use are approaching the market. At least 30 such products have been expressed in plants and 15 are tested in various phases of the clinical trial process (Ma *et al.* 2005, Sauter 2005). Nevertheless, some hurdles are still to overcome, many of which are not technical. Despite the promised benefits, the commercialization of plant-derived pharmaceuticals is thereby mostly challenged by the uncertain regulatory terrain related to their commercial approval.

3.3 Motivations of farmers to adopt GM crops

3.3.1 Losses in arable crops if no crop protection is undertaken

In general, agricultural systems are not "natural" undisturbed ecosystems, and the inherent control mechanisms are often not sufficient to safeguard high crop productivity (Oerke *et al.* 1994, Oerke & Dehne 1997). In order to ensure plant health and crop yield, farmers have to protect plants against animal pests, weeds, fungal and bacterial pathogens and viruses. The ultimate purpose of crop protection is not the elimination of pests, but to reduce crop losses to an economically acceptable level. It has to be differentiated between the potential loss by pests in a *no-control* scenario and the actual loss occurring despite the present crop protection measures. The potential and actual loss rates widely differ between crops (Fig. 3) and regions, mainly depending on the climate, the pests present in these regions, the crop protection measures used, and the overall crop man-
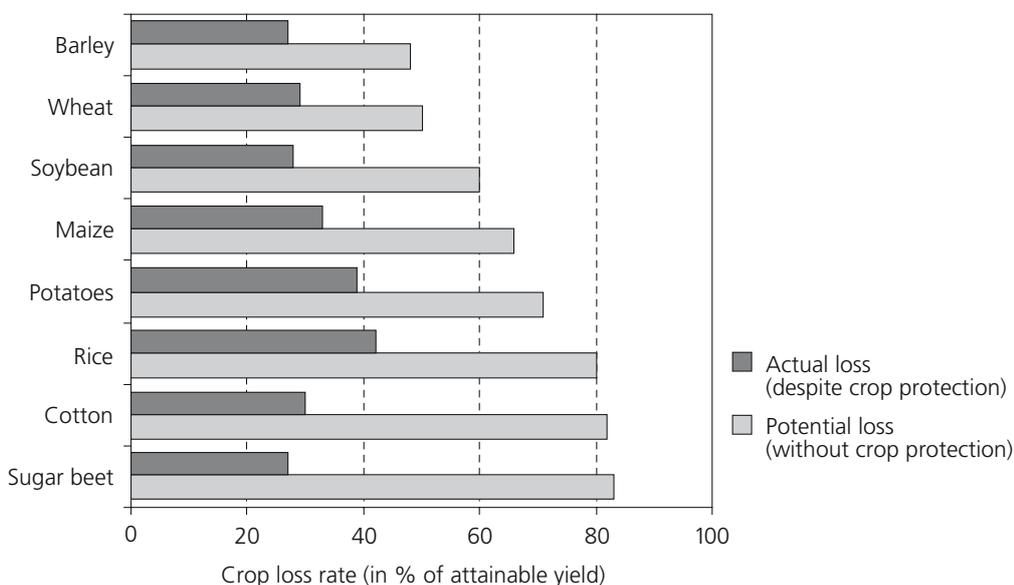
Figure 3: Potential and actual crop losses due to pests, weeds and pathogens in the eight most important arable crops (Oerke & Dehne 2004).

agement. In tropic and sub tropic areas, for example, the potential loss rate in arable crops is highest in cotton and rice. Without any control, worldwide 82% of the attainable cotton harvest and 80% of the rice harvest would be lost. Mechanical, biological and chemical control measures reduce losses to an actual rate of 30% and 42% respectively (Oerke & Dehne 2004). As most crops, cotton production relies heavily upon herbicides to control weeds, often requiring application of two or more herbicides at planting as well as post-emergence herbicides later in the season. Cotton production also uses large amounts of insecticides with 77% of the cotton acreage treated in the U.S. (Fernandez-Cornejo & Mc Bride 2002).

In the Northern Hemisphere, sugar beet often suffers losses from weed competition as the development of seedlings is rather slow and long. Without any crop protection measures sugar beet yields would be reduced by an average of more than 80% in all growing regions (Oerke & Dehne 2004). Actual crop protection practices safeguard more than 56% of the attainable sugar beet production from destruction. In maize, worldwide production is challenged by competition from weeds being the most important pest group (Oerke & Dehne 2004). Losses are effectively reduced under intensive production conditions in large parts of the Northern Hemisphere. In U.S. agriculture, maize is the largest herbicide user with 96% of the acreage treated in the ten major maize producing States (Fernandez-Cornejo & Mc Bride 2002). Due to technical difficulties and to problems in assessing the correct time of spraying, only a small percentage of the maize area is usually sprayed with insecticides against the European Corn Borer (see chapter 10.2.1). Weeds are the predominant pest group in soybean production (Oerke & Dehne 2004). Worldwide, mechanical and chemical crop protection methods are able to reduce the potential loss due to weeds in soybean by more than 70%. In the U.S., for example, more than 97% of the soybean acreage is treated with herbicides.

The primary motivation for farmers to adopt the currently available GM crops is an expected increase in the profitability compared with conventional cultivars. The profitability may vary greatly by region, crop and trait, but also depends on a number of other factors including seed premiums, crop prices and prices of alternative pest control options (Fernandez-Cornejo & Mc Bride 2002). Farmers can benefit from the adoption of GM crops if they are able to spend less money on chemicals and less time and effort applying them. Where GM crops provide more effective protection from pest and weed damage, farmers profit from higher yields and a reduced risk of crop losses. Apart from these direct economic benefits, GM crops may further have indirect benefits for farmers such as improved soil quality due to reduced soil compaction and erosion, reduced water requirements for pesticide spraying, and reduced exposure of farmers and farm workers to pesticides (Brookes & Barfoot 2005).

3.3.2 Herbicide tolerant crops

Herbicide-tolerant crops permit the use of broad spectrum herbicides such as glyphosate (Roundup Ready®) or glufosinate ammonium (Liberty®) at the post-emergence phase. Growers of GMHT crops versus traditional crops benefit mainly from lower costs due to simpler weed control. They expect to achieve at least the same output while lowering weed control costs for chemicals, chemical applications, and tillage (Fernandez-Cornejo & Mc Bride 2002). Cost reductions due to reduced herbicide inputs are probably not the main motivation for farmers to adopt GMHT crops, because most studies support only a small reduction in herbicide use (see 10.1.3). A more important factor believed to influence the economics of adopting herbicide-tolerant crops are the simplicity and flexibility of the weed control program. Herbicide-tolerant programs allow growers to

use one product instead of several herbicides to control a wide range of both broadleaf and grass weeds without sustaining crop injury. In GMHT soybean, for example, glyphosate replaces three or four herbicides, which often control only certain weed groups, and are often applied separately (Gianessi 2005). An example for a country with a rapid and large scale adoption of glyphosate tolerant soybeans is Argentina (see Box 1 for details).

3.3.3 Insect resistant GM crops

Studies on the economic impacts of insect-resistant GM crops are revealing benefits for farmers, most of all where yields are hampered by high pest or weed incidence or where the development of resistant pests impedes the use of pesticides (FAO 2004, Raney 2006). The benefits related to the adoption of *Bt*-crops may comprise both higher yields and significant reductions in pesticide use for some crops (see 11.1). The use of *Bt*-crops can lower costs by reducing the application of insecticides for pest control. Because chemical insecticides are generally not as effective as the control achieved with *Bt*-crops, yield losses are lower in *Bt*-crops than in insecticide treated crops. *Bt*-crops provide a relatively simple and reliable pest control option, because the plant is constantly expressing the insecticidal protein throughout the growing season, whereas the efficacy of insecticide

Box 1: The case of GM herbicide-tolerant soybean in Argentina

GMHT varieties have been rapidly adopted in Argentina - within five years after the first introduction of GM crops, 90% of the total area planted with soybean consisted of GMHT-varieties (Trigo & Cap 2003, Qaim & Traxler 2005). Although yields of GMHT soybean are not significantly different from yields of conventional soybean, the introduction of GMHT soybeans in Argentina had two main advantages: crop management was greatly facilitated and production costs were considerably reduced (Qaim & Traxler 2005). While large-scale farmers were already relying on herbicides as main weed management practice prior to the availability of GMHT crops, many small-scale farmers in Argentina did not apply herbicides, but were using tillage to control weeds. The adoption of GMHT soybean in Argentina has therefore resulted in a net increase in the total volume of herbicides used relative to the amounts used before adoption. This increase should, however, be placed in the context of glyphosate replacing more toxic herbicides. The adoption of GMHT soybean has also increased the area cultivated with conservation tillage practices from 0.3 Mio hectares in 1990 to more than 9 Mio hectares in 2000 (Trigo & Cap 2003) with clear environmental benefits compared to the previously used conventional tilling practice (see 11.2.). The more effective weed management techniques, especially the reduction in energy costs due to less tillage operations, have contributed to cost reductions of approximately US$ 20 per hectare (Trigo & Cap 2003). The reduced work load due to simpler weed management in GMHT crops also resulted in a more flexible and convenient time management, which in turn allowed the cultivation of a second crop in some areas where only one crop was planted before the availability of GM crop varieties (Trigo & Cap 2003). The large adoption of GMHT soybean has, however, also resulted in new land that was taken into agricultural production, the ecological effects of which are hard to evaluate (Qaim & Traxler 2005). This case exemplifies the complexity of economic, environmental and societal interactions of agricultural production systems and new technologies.

treatments is often lowered due to unfavourable weather conditions and difficulties in assessing the right application time. However, because insect infestation can vary considerably each year and the decision to plant *Bt*-crops must be made prior to observing the insect infestation, the farmer may or may not make the most economical decision for a given year depending upon the resulting infestation. *Bt*-crops may act as insurance against significant losses that could occur in the event of severe pest infestation. The most apparent benefits have been associated with the adoption of *Bt*-cotton (Tab. 2). *Bt*-cotton varieties had higher average yields, lower pesticide use and higher net returns than their conventional counterparts in all of the developing countries where studies have been undertaken (FAO 2004, Raney 2006). Although it is too early to assess conclusively the level and stability of yields of *Bt*-varieties compared with conventional varieties, the FAO concludes that the data so far and the rapid pace of adoption suggest that farmers are benefiting from *Bt*-cotton (FAO 2004).

Another motivation for the cultivation of *Bt*-maize is the reduction in mycotoxin contaminations. Fungi of the genus *Fusarium* are common fungal pathogens of maize and

are also known to produce mycotoxins, which may be dangerous for both animal and human health (Bakan *et al.* 2002). The protection of *Bt*-maize against the European Corn Borer results in less insect damage to the maize plant avoiding entry ports for the myco-toxin producing fungi. *Bt*-maize showed to have reduced ear rot and fumonisin contami-nation when compared to non-transgenic maize plants (Munkvold *et al.* 1997, Munkvold *et al.* 1999, Bakan *et al.* 2002). According to Munkvold & colleagues (1999), these results indicate that the use of *Bt*-maize may under some conditions enhance its safety for ani-mal and human consumption.

Table 2: Profit estimates and performance differences between *Bt*- and conventional cotton (adapted from FAO, 2004)

	in	Argentina	China	India	Mexico	South Africa
Yield	(% of kg / ha)	+ 33	+ 19	+ 80	+ 11	+ 65
Gross revenue	(% of $ / ha)	+ 34	+ 23	--	+ 9	+ 65
Chemical sprays	(no.)	- 2.4	--	- 3.0	- 2.2	--
Pest control	(% of $ / ha)	- 47	- 67	--	- 77	- 58
Seed costs	(% of $ / ha)	+ 530	+ 95	--	+ 165	+ 89
Total costs	(% of $ / ha)	+ 35	- 16	--	- 27	+ 3
Profit	**(% of $ / ha)**	**+ 31**	**+ 340**	**--**	**+ 12**	**+ 299**

-- No information available

4 Different risk perceptions of GM crops

The commercial approval of GM crops is among other factors depending on the individual perception of risk by regulatory agencies and on which risk management option is chosen to reduce remaining uncertainties. When judging the risks of GM crops it is important to take into account that the perception of these risks varies considerably both within different stakeholders of the society, but also within different cultural backgrounds. Not surprisingly, the risks of GM crops are not perceived as being uncontrollable in countries where there is commercial cultivation today. This perception is also reflected in the way these countries regulate GM crops and how the risks of GM crops are analyzed. GM crop growing countries obviously trust the risk analysis procedure they apply, and they believe that these procedures allow them to control and manage the risks and uncertainties possibly related to GM crops. When evaluating the available scientific data from commercial cultivation on environmental impacts of GM crops, it is crucial to take into account the risk analysis procedures that are followed in GM crop growing countries and how this has implications for the management of risks and uncertainties.

4.1 General principles of risk analysis

Today, for many risk sources, a risk assessment is a prerequisite to obtain permission to market a product. The principle of assessing risks of chemicals and other stressors to human health and the environment dates back at least to the early 1970s (Suter 1993, Hill & Sendashonga 2003). The National Research Council of the US National Academy of Sciences established a four steps paradigm for risk assessment (*hazard identification, hazard characterisation, exposure assessment and risk characterisation*), which was originally designed for human health assessment, but was later adopted for environmental risk assessment (NRC 1983). Most, if not all of the frameworks for risk assessment, are based on this report. In recent years there has been a wide recognition that dealing with risks should follow a structured approach, described as risk analysis (European Commission 2000b). Although this term does not immediately identify its scope, there is agreement that risk assessment, risk management and risk communication (i.e. the interactive exchange of information and opinions throughout the risk analysis process) are its essential elements (European Commission 2000b; Fig. 4).

Risk assessment and risk management are different processes. Risk assessment is clearly restricted to the phase prior to commercial approval of a product. It intends to evaluate the likelihood of an adverse effect occurring to man or the environment while using this product under a defined set of conditions, together with a value judgement of the results. However, the risk assessors should not have an exclusive role in identifying what is an acceptable risk. This is considered to be part of risk management, which should control an identified risk but also cover possible uncertainties (European Commission 2002). Risk management can therefore be described as the process of weighing policy alternatives in the light of the result of a risk assessment and of other evaluations, and, if required, of selecting and implementing appropriate control options (including monitoring/surveillance activities). A risk/benefit assessment should therefore be common practice in an approval process (European Commission 2000b), where benefits and risks of the product or a technology are weighed by comparing its positive and negative effects with current practice.

Figure 4: The three components of risk analysis constituting the risk cycle (European Commission 2000b).
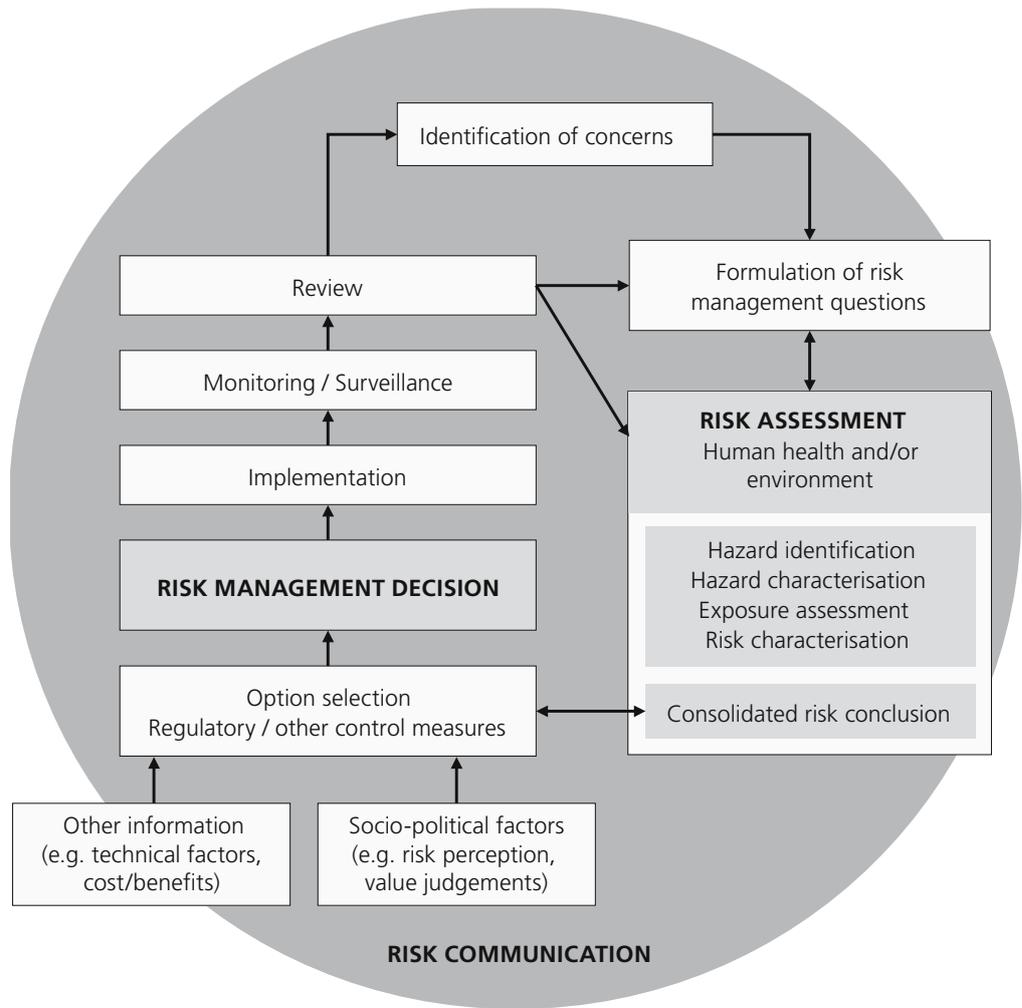
4.2 Risk assessment of GM crops and consequences for risk management

There are several reasons that have lead to the regulation of genetically modified crops. The protection of human health and the environment is the primary reason for government oversight and regulation. There are other factors beside the safety aspect that have supported government decisions to regulate GM crops. Among others, there is the novelty of transgenic crops and the uncertainty accompanying the transformation process, or the public concerns about the safety of transgenic crops (Jaffe 2004). To date, there are no verifiable reports that the cultivation of GM crops has caused significant health or environmental harm in those countries where they are grown (FAO 2004). There is general scientific consensus, however, that individual GM crops could potentially present risks to humans or to the environment (Jaffe 2004) and that the regulation of GM crops is necessary to ensure that potential effects have been assessed on a case-by-case basis before market approval.

4.2.1 The "substantial equivalence" approach

There are two different approaches to regulate GM crops, which follow two rather different concepts (Fig. 5). At the heart of the "substantial equivalence" concept is the approach that a GM crop can be compared to its traditional counterpart that has an established history of safe use. The objective is to determine whether the novel plant presents any new or greater risks in comparison with its conventional counterpart. It is

USA, Canada etc.		Europe
Substantial equivalence As long as no damage can be detected → harmless	⟷	**Precautionary approach** As long as it is not proven that there is no damage → harmful
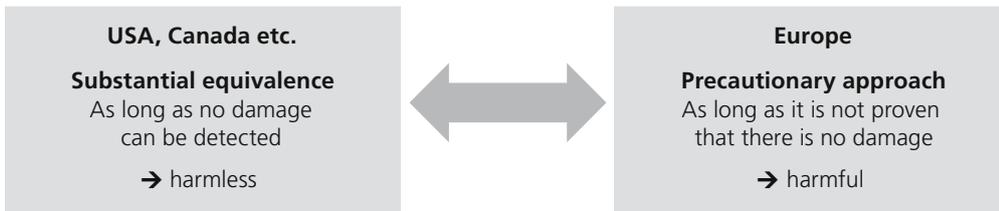
Figure 5: Two main approaches used for the regulation of GM crops in North America and in Europe. The two approaches diverge in their perception of how risks should be managed.

not a risk assessment as such, but a way of structuring the comparison to identify any differences that then become the focus of the risk assessment. For example a transgenic insect-resistant maize plant is first and foremost a maize plant, and the goal is to evaluate what, if any, additional risks to human health or impacts on the agro-ecosystems may result from the incorporation of the new trait.

The rationale of the "substantial equivalence" concept states that products that received regulatory approval are considered to present no more risks than comparable products with a history of safe use. Post-market monitoring activities are therefore often not judged necessary or are limited to very specific areas, such as insect resistance monitoring of *Bt*-maize cultivation, as in the United States (Jaffe 2004). However, in the United States and in Canada, it is the developer's (i.e. the seed company) responsibility to monitor for unintended or unexpected environmental effects following the release of its new GM crop. The developer must inform the regulatory authorities, such as the U.S. EPA or the Canadian Food Inspection Agency (CFIA) of any new information regarding the risks to the environment (e.g. enhanced weediness characteristics) or to human health (e.g. exposure to allergens) resulting from the release of the new GM crop. On the basis of the new information, the authorities will re-evaluate the potential risk to the environment, including the potential risk to human health posed by the release. The authorities may maintain, change, or remove existing conditions respecting the release, impose additional conditions, or refuse or cancel the authorization and require the applicant to stop the release and take any appropriate action necessary to eliminate or minimize the risk.

4.2.2 The precautionary approach

The second concept is the "precautionary approach", which in the European Union should be taken into account when introducing GM crops into the environment (Euro-

Pre-market risk assessment (PMRA)	Post-market monitoring (PMM)
Surveillance before GMP commercialisation (e.g. biodiversity monitoring)	General surveillance

Risk assessment in laboratory/greenhouse → Decision based on ERA I → Risk assessment in field trials → Decision based on ERA II → Case-specific monitoring → Decision based on ERA III

Risk assessment if needed

Containment → Limited release → Commercial cultivation → time

Approval for limited release — Approval for placing on the market — Renewal of consent

Figure 6: Stepwise procedure of ecological risk assessment during the life cycle of a specific GM crop (Sanvido *et al.* 2005). (ERA= environmental risk assessment).

pean Community 2001). The precautionary approach states, that "when an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically" (Raffensberger & Tickner 1999). Therefore, the introduction of GM crops into the environment should generally be performed according to the step-by-step principle, which means that GM crop releases can only be scaled if a risk assessment based on information of the preceding step has resulted in an acceptable risk estimation for the next step (European Community 2001; Fig. 6).

4.2.3 Residual uncertainty – debating the limits of the precautionary approach

The precautionary approach is closely linked to the obligation of proof (van den Belt 2003). "The applicant or proponent of an activity or process or chemical needs to demonstrate that the environment and public health will be safe" (Raffensberger & Tickner 1999). A strong application of the precautionary principle would signify that every technology, such as genetic engineering of agricultural crops, has to be considered unsafe until it is proven safe. However, this assumption is flawed, because the proof of absolute safety is an impossible task (van den Belt 2003). The environmental risk assessment can not give an absolute proof on the inoffensiveness of a GM crop, since uncertainty is inherent to the concept of risk (Hill & Sendashonga 2003).

Sources of uncertainty in risk analysis

Risk assessments are always framed by some uncertainties (Levidow 2003). The first step in quantifying risk is to identify the sources of uncertainty, which are inherent to every risk analysis procedure. Scientists seek to inform policy makers with simple and clear advice, albeit they will often have to rely on results of complex studies that predict uncertain outcomes (Harwood & Stokes 2003). Although these uncertainties will not be quantifiable in most cases, it is important to know the sources of uncertainty when considering risks, costs and benefits of a technology. Four different sources of uncertainty can be identified (Harwood & Stokes 2003):

(1) Process stochasticity as a consequence of random behaviour of systems that have chaotic dynamics. It is also referred to as natural variation of ecosystems,
(2) Observation or measurement errors due to the sampling strategies used,
(3) Model error – all models are imitations of reality since there is a need for extrapolation based on limited information and data,
(4) Policy and market forces – implementation errors due to human misbehaviour must be taken into account for managed systems.

There are mainly four options how to deal with uncertainties:
- perform further research,
- adopt risk management measures, such as e.g. mitigation measures, or environmental monitoring,
- accept uncertainties as not being resolvable,
- weigh risks with possible benefits that could result from the adoption of the technology.

The Cartagena Protocol on Biosafety explicitly recognizes that scientific uncertainties exist and that decisions must be taken recognizing that those uncertainties may not be resolved (CBD 2000). This is also recognized by the European Commission, which states that the precautionary principle is particularly relevant to the management of risks (Euro-

pean Commission 2000a) and risk management should control an identified risk and cover the uncertainties (European Commission 2002). In order to detect changes in the environment related to GM crops, monitoring of the commercial cultivation is an appropriate measure to apply the precautionary principle (Fig. 6). A consistent application of the precautionary principle would in the final analysis stifle all innovations (van den Belt 2003). To ensure that a policy is truly precautionary one should compare the risks of adopting the policy against the risks of not adopting it (Goklany 2002). It is interesting to notice that neither the EU nor the Swiss legislation, both by following the precautionary approach, do consider possible benefits for the approval of GM crops. Only potential adverse effects on human health and the environment are evaluated, although a risk/ benefit assessment should be common practice in an approval process, as it is common for many other hazards (European Commission 2000b). Unfortunately, none of the versions of the precautionary approach provides any guidance on how it should be applied if a technology might have both positive and negative outcomes and where both set of outcomes are uncertain (Goklany 2002). We believe that the approval process for commercial cultivation of a GM crop should include a risk/benefit assessment where the benefits and risks are weighed by comparing positive and negative effects of the GM crop system with current agricultural practices.

Summary: Principles of regulation in GM crop growing countries

- A risk assessment is a prerequisite to obtain permission to market any GM crop variety. GM crop growing countries generally follow the substantial equivalence approach, which states that a GM crop should be compared with its traditional counterpart that has an established history of safe use.

- GM crop varieties that received regulatory approval are considered to present not more risks than comparable conventional varieties with a history of safe use. In the U.S. and in Canada, it is the seed company's responsibility to monitor for unintended or unexpected environmental effects following the release of its new GM crop variety and to inform the regulatory authorities of any new information regarding the risks to the environment.

- European countries follow the "precautionary approach", which states that the introduction of GM crops into the environment should only be performed if a risk assessment has determined that the risk is acceptable. GM crops are therefore basically considered being unsafe until they are proven to be safe.

- Risk assessments are always framed by some uncertainties. The Cartagena Protocol on Biosafety explicitly recognizes that scientific uncertainties exist and that decisions must be taken recognizing that those uncertainties may not be resolved. Post-market environmental monitoring during commercial cultivation of GM crops is an appropriate measure to apply the precautionary approach.

5 GM crops, modern agricultural systems and the environment

Potential impacts of GM crops should be put in relation to the environmental impacts of modern agricultural practices that took place during the last decades. Modern agricultural systems have an impact on all environmental resources, including soil fertility, water (applications of fertilizers and pesticides increasing nutrient and toxins in ground and surface waters), and air (nitrogen fertilization increasing the emission of greenhouse gases and contributing to global warming) (Tilman *et al.* 2002). Within the scope of this review, we will, however, concentrate on discussing potential impacts on biodiversity.

5.1 Environmental impacts of modern agricultural systems

Modern agricultural systems have considerable negative impacts on global biodiversity (Chapin *et al.* 2000, Stoate *et al.* 2001, Hails 2002, Robinson & Sutherland 2002, Tilman *et al.* 2002, Ammann 2005). On a global scale the most direct negative impact is due to the considerable loss of natural habitats, which is caused by the conversion of natural ecosystems into agricultural land (McLaughlin & Mineau 1995, Chapin *et al.* 2000). The negative impact of modern agricultural systems in Europe can not be ascribed to only one factor, but was caused by the interaction of a multitude of factors. Several changes in the management of agricultural land over the last century have resulted in a decline in the diversity of plant, invertebrate and bird species within agro-ecosystems. The significant decline in floral diversity of grasslands and arable field margins was mainly caused by high yielding forage crop varieties, increased fertilizer inputs, frequent applications of herbicides and the increased purity of crop seed (Hails 2002, Walter *et al.* 2005). Modern agricultural systems have produced a landscape in which many fields have very few weeds and very few invertebrates providing little food for birds. This shift in the type and density of weeds in the fields, as well as the disappearance of important habitats such as large stretches of hedgerows, was mainly responsible for the dramatic decline in bird populations (Chamberlain *et al.* 2000, Robinson & Sutherland 2002, Royal Society 2003). Two studies from the United Kingdom, for example, concluded that negative biodiversity impacts were mainly due to an increase in intensification and specialisation of farming, with a move from mixed farming to a predominance of arable farming and grassland (Marshall *et al.* 2003, Royal Society 2003). The authors of these studies identified two major influential factors: (1) the switch from planting spring cereals to planting autumn cereals, and (2) the move from cutting grass for hay to the production of silage for animal fodder, meaning earlier and more mowing, reseeding, fertilising and tillage. Although no similar data is available for Switzerland, it can be assumed that similar conclusions are also valid for Swiss agriculture.

5.2 Potential environmental impacts of GM crops

Potential environmental impacts of the currently commercialized GM crops can roughly be subdivided into direct and indirect effects (Wolfenbarger & Phifer 2000, Pretty 2001, Dale *et al.* 2002, Conner *et al.* 2003, Snow *et al.* 2005). Direct effects could result from the particular nature of the genetic change, i.e. from the resulting genotype and phenotype of the crop modified (Fig. 7). GM crops could be able to hybridize with sexually compatible wild relatives and these could subsequently suffer an increased risk

ECOLOGICAL EFFECTS

Direct effects

Gene flow to wild relatives	Invasiveness of GM crops	Environmental fate of transgenic products	Effects on non-target organisms	Resistance development
Pollen transfer to wild relatives and hybrid formation	Survival outside of cultivated areas	Persistence, degradation and spread of transgenic products	Direct or indirect uptake of transgenic products through feeding	Resistance development in target organisms
Survival and reproduction of hybrids	Reproduction outside of cultivated areas	Accumulation of transgenic products in soil	Effects on non-target organisms	Indirect effect
Transgenic population (hybrids / crops) with enhanced fitness compared to wild population		Eluviation of transgenic products from soil	Effects on population dynamics	
Spread and persistence of transgenic plants (hybrids / crops) outside of cultivated area		Immission of transgenic products into water	Effects on ecosystem-functions	

Indirect effects

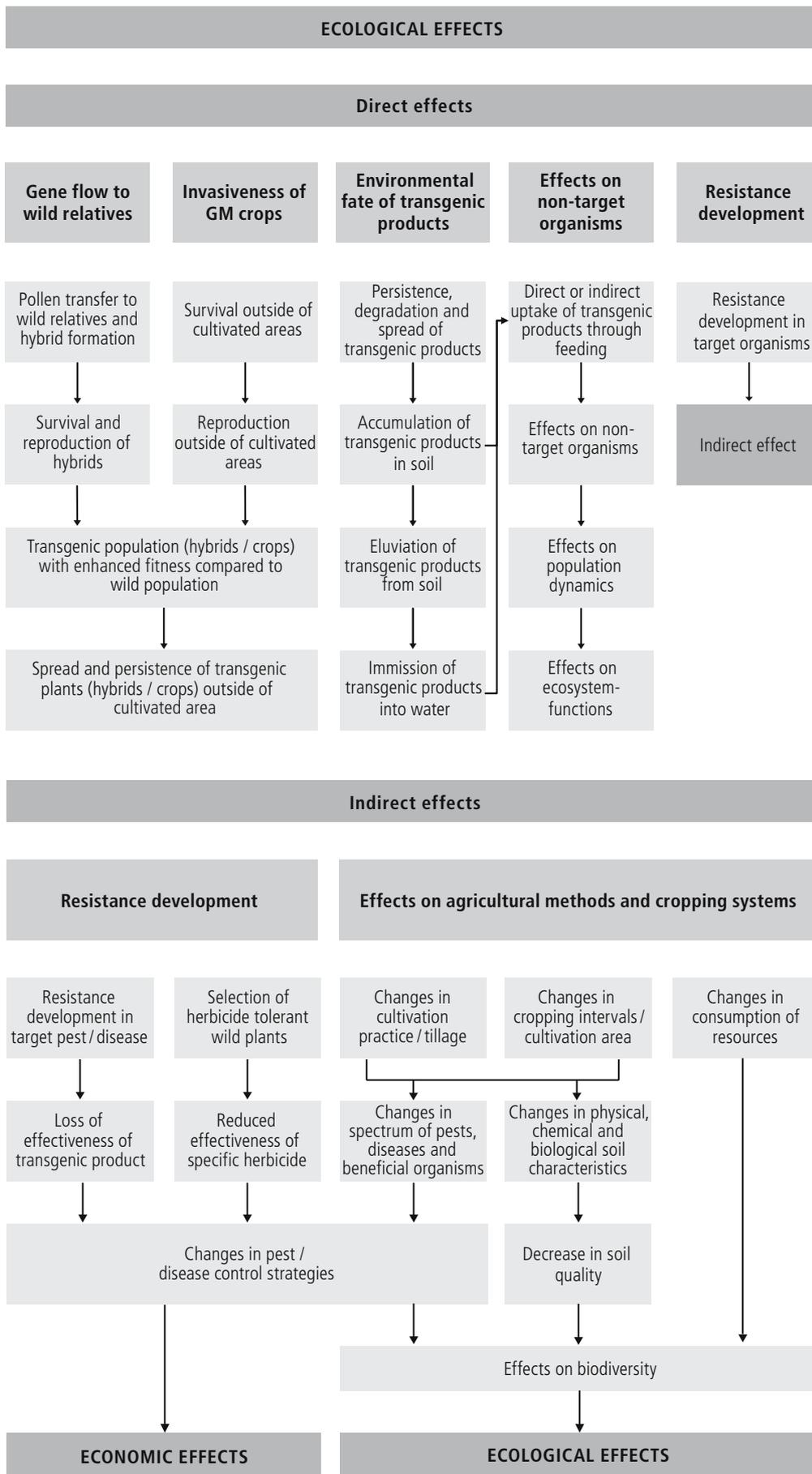| Resistance development		Effects on agricultural methods and cropping systems		
Resistance development in target pest / disease	Selection of herbicide tolerant wild plants	Changes in cultivation practice / tillage	Changes in cropping intervals / cultivation area	Changes in consumption of resources
Loss of effectiveness of transgenic product	Reduced effectiveness of specific herbicide	Changes in spectrum of pests, diseases and beneficial organisms	Changes in physical, chemical and biological soil characteristics	
	Changes in pest / disease control strategies		Decrease in soil quality	
		Effects on biodiversity		

ECONOMIC EFFECTS   **ECOLOGICAL EFFECTS**

Figure 7: Potential direct and indirect effects of genetically modified crops on the environment (adapted from Wolfenbarger & Phifer 2000, Dale *et al.* 2002).

of extinction. Introduced genetically modified traits could make a crop more likely to be more persistent (weedy) in agricultural habitats or more invasive in natural habitats. Transgenic products, especially toxins produced to be active against certain pests, could be harmful to organisms that are not intended to be harmed. Target organisms could develop resistances against the insecticidal proteins produced in GM crops resulting in a loss of effectiveness of the transgenic product. Changes in the agricultural practice due to the adoption of GM crops (e.g. soil tillage, cropping intervals, or cultivation area) could result in indirect effects (Fig. 7).

5.2.1 Unintended effects of GM crops

Recombinant DNA methods have been viewed as particularly precise because the inserted gene sequences can be characterized and monitored. Nevertheless, some authors have raised concerns that the transformation process could result in various unintended effects, which are unrelated to the nature of the specific transgene (Wilson *et al.* 2004, Birch & Wheatley 2005, Snow *et al.* 2005). Unintended phenotypes could be caused by the random insertion of transgenic sequences into chromosomal locations, often at multiple sites in the genome, and the random insertion could lead to an alteration of primary and secondary plant metabolite processes. Several authors, in contrast, have also stated that the occurrence of unintended effects is not a phenomenon specific to genetic engineering (Cellini *et al.* 2004, Snow *et al.* 2005). There is no indication that unintended effects are more likely to occur in GM crops than in conventionally bred crops (Cellini *et al.* 2004). Unwanted health and plant disease risks have, for example, also arisen in conventionally bred celery, potato and maize through the appearance of toxic compounds (NRC 2000). Breeding procedures should, however, prevent the occurrence of such unintended effects in most cases. The common practice of any breeding program is to select favourable lines and to discard those lines exhibiting unwanted properties. Abnormal individuals or their progeny are eliminated during extensive screening among multiple locations and years (Cellini *et al.* 2004, Snow *et al.* 2005). Likewise their non-transgenic counterparts, it is most unlikely that GM crops with obvious abnormalities will be used in commercial lines.

The safety of GM crops is better characterised than conventionally bred crops, including knowledge on the site and nature of the genetic modification (Cellini *et al.* 2004). In addition to the classical breeding process, the introduction of crops produced by genetic engineering is additionally regulated by a thorough pre-market risk assessment of potential unwanted effects of the GM crop on the environment (European Community 2001). It is common practice today to perform a large number of so-called targeted analyses to demonstrate that the characteristics of a novel GM crop are comparable with those of the conventional counterpart. These analyses include key macronutrients, micronutrients, antinutrients and toxins (Lehesranta *et al.* 2005). A comparative assessment should always consider the extent of natural variation and not simply compare GM lines and parental controls. A comparative analyses assessing the proteome diversity of a range of non-GM potato (*Solanum tuberosum*) germplasm and eight GM lines of potato indicated that the variation between the non-GM cultivars was much greater than the differences between the GM lines (Lehesranta *et al.* 2005). There were considerably fewer differences between the GM and non-GM lines of the same genetic background than between different non-GM cultivars. These results were confirmed by another study, where the total metabolites of several field-grown GM potato lines were compared to conventional potato cultivars (Catchpole *et al.* 2005). Apart from targeted changes, the GM potatoes appeared substantially equivalent to traditional cultivars, but a large variation was found in the metabolite profile of the conventional cultivars.

Summary: GM crops, modern agricultural systems and the environment

- Independent from the use of GM crops, modern agricultural systems have profound impacts on all environmental resources, including considerable negative impacts on global biodiversity. Several changes in the management of agricultural land over the last century have resulted in a decline in the diversity of plant, invertebrate and bird species within agro-ecosystems. Modern agricultural systems have produced a landscape in which many fields have very few weeds and very few invertebrates providing little food for birds.
- The safety of GM crops is better characterised than conventionally bred crops. In addition to the selection process performed during classical breeding, the introduction of crops produced by genetic engineering is additionally regulated by a thorough pre-market risk assessment of potential unwanted effects of the GM crop on the environment.

6 Effects of GM crops on non-target organisms

For the currently commercially cultivated GM crops, it is generally accepted that toxic effects on non-target organisms are restricted to GM crops expressing insecticidal proteins (Wolfenbarger & Phifer 2000, Dale et al. 2002, Conner et al. 2003). The following chapter is therefore focussing on effects, which are related to a transgenic product, further dividing them in direct and indirect toxic effects (see chapter 6.1.1). When discussing potential effects of transgenic crops on non-target organisms it is important to distinguish between effects related to a transgenic product and those occurring independently from a transgenic product (see Box 2 for details). Herbicide tolerant crops are considered to have no direct toxic effects on non-target organisms, because the enzymes conferring the herbicide tolerance are normally expressed in plants and they are not known to have any toxic properties (APHIS-USDA 1994, Carpenter 2001). The use of herbicide tolerant crops could, however, result in indirect environmental effects caused by changes in the agricultural practice. Because this category of indirect effects occurs independently from a specific toxin it will be discussed in chapter 10.

Box 2: Glossary of terms "Effects of GM crops on non-target organisms"

Effects related to a transgenic product		Effects occurring independently from a transgenic product	
Direct toxic effects	Indirect toxic effects	Unintended effects due to the genetic modification	Indirect effects due to changes in the agricultural practice

Direct toxic effects are caused by biologically active compounds (e.g. the *Bt*-toxin) and do only result if a specific mode of action of the toxin is taking place in the organism.

Indirect toxic effects are caused in natural enemies by changes in the availability and/or the nutritional quality of target herbivores as prey[1] items.

[1] The terms "prey" (related to predators) and "host" (for parasitoids) are considered as being equivalent

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6.1 Potential non-target effects of insect-resistant plants

There are concerns that insect-resistant GM crops could harm organisms other than the pest(s) targeted by the toxin. Insect resistance conferred via expression of Cry-proteins from *Bacillus thuringiensis* (*Bt*) is by far the most common trait that has been engineered into plants. To date *Bt*-toxins[1] represent the only insecticidal proteins expressed in commercial GM crops (James 2005, AGBIOS 2006). Other insecticidal proteins, like protease inhibitors (PIs) and lectins, have been engineered into different crops, but they have remained in the experimental stage until now (Jouanin *et al.* 1998). Their ranges of insecticidal activity are generally broader than those of *Bt*-toxins. Since they have not obtained commercial approval to date, this chapter will concentrate on the currently commercially available *Bt*-crops, focusing primarily on *Bt*-maize expressing the lepidopteran-specific Cry1Ab protein.

[1] The term "*Bt*-toxin" is only referring to Cry-proteins

6.1.1 Defining effects to non-target organisms

In order to estimate if GM crops pose a direct threat to ecological interactions, it is crucial to compare the GM crop system to current crop protection systems. There is a need to define which organisms should not be affected by the cultivation of the GM crop because they are considered ecologically relevant. Non-target organisms are by definition those organisms, which are not the intended target of a particular use of a pesticide (van Leeuwen & Hermens 1995) or similarly a GM crop. From an agronomic point of view, one could also describe non-targets as any wildlife associated with the crop that does not cause economically relevant levels of damage.

We believe that when considering and discussing potential toxic effects of GM crops to non-target organisms, it is important to take into account that there exist different types of effects (Box 2). Direct toxic effects are clearly due to toxic substances in the plant and are depending on a definite mode of action taking place in the organism. Indirect toxic effects, on the other hand, are caused by changes in the availability and/or the quality of the prey consumed.

6.1.2 Ways of exposures of non-target organisms to insecticidal proteins

Non-target organisms have to ingest insecticidal proteins expressed in GM crops in order to be directly affected. Ingestion can occur via several ways of exposures (Fig. 8). Exposure can either occur by feeding on plant material (e.g. leaves, pollen), by feeding on insects that have previously fed on GM crops (and therefore contain the toxin) or via exposure through the environment, e.g. when toxins from plant residues persist in the soil (Groot & Dicke 2002). Exposure via target herbivore(s) may be restricted, because they may not be available due to an almost complete control of the target pest(s) (e.g. *Ostrinia nubilalis* in *Bt*-maize expressing Cry1Ab). Each type of exposure has to be evaluated according to its ecological relevance since each type depends on various factors including feeding behaviour of both herbivores and entomophagous arthropods, availability of prey, and expression of the insecticidal protein in different plant tissues (Dutton *et al.* 2003). Exposure via the environment is discussed in chapter 7.
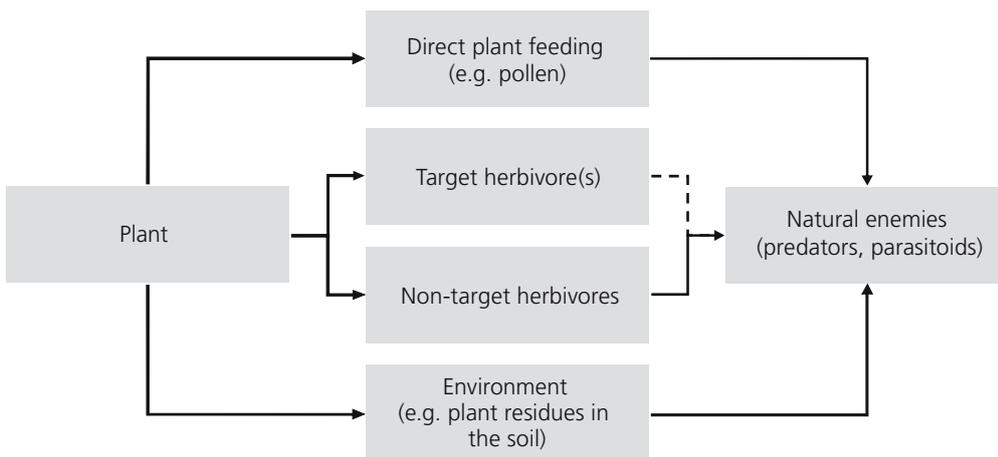
Fig. 8: Routes of exposure of plant-produced insecticidal proteins to arthropods of different trophic levels.

Some authors argued that the constitutive expression of the *Bt*-toxin in all plant tissues throughout the growing season leads to continuous exposure of non-target organisms to the insecticidal protein and thus increase the risk of non-target effects (Hilbeck 2001, Birch & Wheatley 2005). However, other authors considered the constitutive expression of the *Bt*-toxin as a potential advantage because this would actually reduce

the environmental exposure of non-target organisms to the toxin (Poppy & Sutherland 2004). Only those insects consuming parts of the plant expressing the toxin are exposed, whereas with conventional insecticide spraying or *Bt*-spray formulations much more insects are accidentally exposed.

Several authors have recognized that the terms "risk" and "hazard" have often been mixed up, and that this has led to considerable scientific and public misconception (Berenbaum 2001, Shelton & Sears 2001, Gatehouse *et al.* 2002). We therefore think that it is essential to differentiate between studies, which have assessed the mere toxicity (= hazard) and studies, which also took into account realistic levels of exposure of non-target arthropods to the insecticidal protein. A hazard to a single organism may be possible due to a toxic substance, but this hazard may not be relevant in an ecological context where the majority of the population will most probably not be exposed to the insecticidal protein or the exposure is negligible compared to other hazards, which are more likely to occur (e.g. insecticide use or predation).

6.1.3 Assessing the risks of insect-resistant GM crops to non-target organisms

Several authors have proposed procedures on how to assess the risks that insect resistant GM crops may pose to non-target organisms (Cowgill & Atkinson 2003, Dutton *et al.* 2003, EFSA 2004, Poppy & Sutherland 2004, Rose 2006). Although there are differences in these proposals, there is consensus on the fact that non-target risk assessments should include both the factors "hazard" and "exposure" and that a stepwise or tiered approach should be followed. This approach corresponds to the procedures generally used for ecological risk assessment (EPA 1998) and is similar to that employed in pesticide toxicity testing (Hill & Sendashonga 2003). Non-target risk assessments should include the identification of hazards that GM crops could pose to non-target organisms, the determination of the levels at which non-target organisms are exposed to the insecticidal protein, and the assessment if this hazard constitutes a risk.

A stepwise risk assessment approach typically consists of a first step identifying potential hazards and a subsequent step assessing likely exposure levels:

Lower tier studies: hazard identification and impact studies involving multi-trophic effects

Worst-case studies allow to determine whether non-target organisms are susceptible to the insecticidal protein and whether direct toxic effects do occur. Hereby, test organisms are either fed with defined quantities of pure insecticidal protein incorporated into appropriate artificial diets or with transgenic plant material. The use of pure insecticidal protein allows to conduct dose-response tests by using higher insecticidal protein doses than those that are normally occurring in the plant. Most often these studies are conducted in the laboratory during early stages of the approval process. They are relatively simple in design, well controlled, repeatable, and the results are easy to interpret (Dutton *et al.* 2003, Romeis *et al.* 2006). Typically, lower-tier studies are conducted on a restricted number of appropriate surrogate species, since testing of all species that could potentially be exposed to the insecticidal protein is not practicable. Multitrophic studies involve plants, herbivores feeding on plants, and predators or parasitoids using the herbivores as their prey. This approach is used to investigate effects of the GM crop on organisms that are not directly exposed but one or two steps higher up in the food chain. Such studies are often initiated by earlier test results where hazards can not be excluded and uncertainties have to be reduced.

Higher tier studies: exposure studies under field conditions

If lower tier studies were not able to sufficiently address questions of particular risk, the last step in a tiered risk assessment would include field studies. To assess direct toxic effects of an insecticidal protein to non-target organisms it is important to consider the degree of exposure to the transgene product. Field trials simulate the cultivation of GM crops in order to quantify actual levels of exposure of different species and to determine likely ecological effects due to the GM crop and its management. In contrast to lower tier studies conducted in the laboratory and in the greenhouse, one could think that field studies should by definition be more suitable to consider the ecological context of agricultural practice. However, due to the complex ecological interactions occurring in such experiments, it is often difficult to unambiguously associate effects to a specific cause, i.e. to clearly determine if effects can be associated to the GM crops and/or its management.

6.2 Insect-resistant *Bt*-crops

6.2.1 *Bacillus thuringiensis (Bt)*

Bacillus thuringiensis (*Bt*) is an endospore-forming soil bacterium characterized by the presence of protein crystals (Cry-proteins) within the cytoplasm of the sporulating cell. The different strains of *B. thuringiensis* contain varying combinations of Cry-proteins (so-called *Bt*-toxins) and each of these insecticidal proteins is known to have a very selective toxicity against different groups of arthropods. They specifically bind to receptors in the midgut causing the formation of lytic pores in the epithelial cell membrane leading to the death of the insect (Höfte & Whiteley 1989, Schnepf *et al.* 1998, de Maagd *et al.* 2001). Microbial insecticides containing Cry-proteins have commonly been used since the 1950s for insect control in *Bt*-spray formulations. Due to their high specificity, *Bt*-sprays are regarded as environmentally friendly and their widespread use has shown to have only few adverse effects on non-target organisms and human health (Glare & O'Callaghan 2000). Currently, *Bt*-sprays comprise one to two percent of the global insecticide spray market, and half of the current sales are used in Canadian forests to control gipsy moths, spruce budworm and other lepidopteran pests (Nester *et al.* 2002, Shelton *et al.* 2002).

6.2.2 Genetically modified *Bt*-crops

Insect resistance conferred via expression of various Cry-proteins from *B. thuringiensis* is by far the most common insecticidal protein that has been engineered into plants and is up to now the only one that is commercially used in GM crops (James 2004). *Bt*-genes have been engineered into a large number of plant species such as maize, cotton, potato, tomato, rice, eggplant and oilseed rape (Ely 1993, Shelton *et al.* 2002, de Maagd 2004). However, at present, genetically modified *Bt*-maize and *Bt*-cotton are the only crops that are commercially cultivated. Transgenic *Bt*-potato plants expressing Cry3Aa to control the Colorado potato beetle (*Leptinotarsa decemlineata*) were commercialized from 1996 to 2001, but were withdrawn from the market due to lack of consumer acceptance and the introduction of a novel insecticide able to control the beetle as well as aphids (Shelton *et al.* 2002). *Bt*-maize expressing Cry1Ab was initially developed to control a lepidopteran pest, the European Corn Borer (*Ostrinia nubilalis*), but has shown to be also effective against various other lepidopteran pests, such as e.g. *Sesamia nonagrioides*, *Spodoptera littoralis* and *Helicoverpa zea* (Pilcher *et al.* 1997, Gonzales-Nunez *et al.* 2000, Dutton *et al.* 2005). *Bt*-maize expressing the beetle-specific Cry3Bb toxin to control corn rootworms (*Diabrotica* spp.) has received commercial approval in 2003 in the United States and in Canada (Ward *et al.* 2005, AGBIOS 2006). However, due to its recent approval, no experience from commercial cultivation is available yet.

6.3 Effects of *Bt*-crops expressing Cry1-proteins on non-target arthropods

The long-term and wide-scale use of *Bt*-crops during the past ten years has been accompanied by extensive studies testing potential adverse effects of these crops. Most studies focused on *Bt*-crops expressing Cry1-proteins and, due to their selective toxicity on moths and butterflies (Lepidoptera), on assessing potential adverse effects on arthropods. Tested species have been selected according to several criteria. Typically, they were selected according to their importance in providing relevant ecological functions in crops, such as natural regulation of pests, as well as bees and other pollinating insects that are often essential for high yields in crop production. However, the selection was also driven by social, ethical and economic factors. Certain species, such as butterflies, may to a certain degree have been selected because of their perceived value for the society. In addition to the above mentioned criteria, test species should be available in reasonable quantities, and amenable for testing (Dutton *et al.* 2003).

6.3.1 Effects on beneficial insects (predators and parasitoids)

A lot of attention has been paid in recent years to investigate potential adverse effects of *Bt*-crops expressing Cry1-proteins on beneficial insects such as predators and parasitoids (O'Callaghan *et al.* 2005, Romeis *et al.* 2006). Predators and parasitoids (so-called natural enemies) are important regulators of insect pest populations, playing a vital role in biological control.

Results of lower tier studies

Prior to the registration of the first *Bt*-plants in the United States in 1995, EPA conducted environmental risk assessments for all Cry-proteins expressed in *Bt*-potato, *Bt*-maize, and *Bt*-cotton. They evaluated studies of potential effects on a wide variety of non-target organisms that might be exposed to *Bt*-proteins, amongst others also different beneficial insects. The results of the laboratory tests showed that direct feeding of purified Cry1-proteins was not toxic to none of the evaluated beneficial insects (EPA 2001).

The risk of *Bt*-maize to natural enemies has further been assessed in several studies involving different trophic levels. Eleven studies have investigated the effects of *Bt*-crops on predators in a tritrophic plant-herbivore-predator system (Romeis *et al.* 2006). Adverse effects on mortality, longevity or development of the predators were only reported in studies using *Bt*-susceptible lepidopteran larvae as prey that had ingested the *Bt*-toxin. In particular, the green lacewing (*Chrysoperla carnea*), an important predator in many maize growing areas, has thoroughly been studied since studies suggested that this predator was negatively affected by Cry1Ab (see Box 3 for details). Results of subsequent studies, however, showed that the insecticidal protein itself does not directly affect this predator, but that the green lacewing may be affected when feeding on *Bt*-susceptible prey species representing a suboptimal food source (Dutton *et al.* 2002, Romeis *et al.* 2004, Rodrigo-Simon *et al.* 2006). The negative effect observed in the third trophic level was entirely prey-quality mediated and caused by altered food quality of lepidopteran larvae that were susceptible to the insecticidal protein they had ingested. Because lepidopteran larvae are not considered an important prey for *C. carnea*, *Bt*-maize poses only a negligible risk for this important predator (Dutton *et al.* 2003, Romeis *et al.* 2004).

The effects of *Bt*-crops on hymenopteran parasitoids developing in herbivores reared on transgenic plants have been investigated in ten studies (Romeis *et al.* 2006). Effects

Box 3: Effects of *Bt*-maize on the green lacewing *(Chrysoperla carnea)*

There have been publications proposing that transgenic *Bt*-maize expressing Cry1Ab may create negative impacts on larvae of the beneficial green lacewing *(Chrysoperla carnea)* when feeding on pests that had ingested the insecticidal protein (Hilbeck *et al.* 1998a, 1998b, 1999). Hilbeck *et al.* reported slightly elevated mortality and prolonged development time in lacewing larvae reared on *Bt*-maize lepidopteran larvae. However, later tritrophic feeding studies with the green lacewing using several different prey species reared on Cry1Ab-maize suggested that the toxin itself does not affect the predator, but that the observed effects were caused by the suboptimal food quality of the *Bt*-susceptible prey used. Effects on lacewing larvae were only observed when they were fed with *Bt*-maize fed *Spodoptera littoralis* larvae as prey but not when fed with *Bt*-maize fed spider mites or aphids (Dutton *et al.* 2002). These results can be taken as an indication for the occurrence of indirect toxic effects since spider mites were found to contain concentrations of active *Bt*-toxin that were more than five times higher than those found in *S. littoralis* larvae (Dutton *et al.* 2002, Obrist *et al.* 2006). Larvae of *S. littoralis* were also found to be sublethally affected by the *Bt*-toxin, suggesting that *S. littoralis* larvae represented a low food quality for *C. carnea* when compared to spider mites or aphids (Dutton *et al.* 2002, 2005). This hypothesis was further supported by the finding that there were no direct effects of the *Bt*-toxin on *C. carnea* when larvae were fed with high doses of pure Cry1Ab toxin (Romeis *et al.* 2004, Rodrigo-Simon *et al.* 2006). This can be explained by the fact that Cry1Ab does not show specific binding to receptors in the midgut of *C. carnea* larvae (Rodrigo-Simon *et al.* 2006). Additionally, when *C. carnea* has the possibility to choose its prey in the field, exclusive feeding on lepidopteran larvae is unlikely as these do not represent a preferred prey and often escape predation (Dutton *et al.* 2003). Furthermore, when given a choice, the predator larvae prefer non-*Bt*-maize fed *S. littoralis* larvae over sublethally affected *Bt*-maize fed larvae (Meier & Hilbeck 2001). The results of the available laboratory and glasshouse studies indicate that *Bt*-maize poses a negligible risk for *C. carnea*, a conclusion which has been confirmed by a number of field studies (Bourguet *et al.* 2002, Candolfi *et al.* 2004, de la Poza *et al.* 2005, Pilcher *et al.* 2005).

on mortality, development, weight or longevity were only observed in cases where *Bt*-susceptible herbivores were used as hosts. This is not surprising given that host-parasitoid relationship is usually tight and parasitoids are very sensitive to changes in host quality.

Field studies

Although lower tier studies have not detected any unexpected effects on natural enemies that would have initiated an immediate need for detailed ecological studies, more than 50 field experiments, varying greatly in size, duration and sampling efforts, have been conducted (Romeis *et al.* 2006). Most studies assessed abundance of natural enemies using different methods, while only few studies compared biological control functions of natural enemies in both *Bt*- and conventional crops.

Experimental field studies have only revealed minor, transient or inconsistent effects of *Bt*-crops when compared to a non-*Bt* control (Eizaguirre *et al.* 2006, Romeis *et al.* 2006). Exceptions were observed with specialist natural enemies which were virtually absent in *Bt*-fields due to the lack of target pests as prey or hosts (Riddick *et al.* 1998, Pilcher *et al.* 2005). Such negative effects on natural enemies that depend on the target pests are a common consequence of every pest control method, including insecticides, biological control, and conventional host-plant resistance and are generally not consid-

ered as a risk (Romeis *et al.* 2006). Three studies in *Bt*-crops revealed consistent reductions in the abundance of different generalist predators that were also associated with the reduced availability of lepidopteran prey (Daly & Buntin 2005, Naranjo 2005a, Whitehouse *et al.* 2005). A six-year field study in *Bt*-cotton on the abundance of 22 arthropod natural enemy taxa indicated that an average decrease of about 20% in some predatory species did not seem to be ecologically relevant for the biological control function of the natural enemy community (Naranjo 2005a, Naranjo *et al.* 2005).

A number of experimental field studies have included conventional insecticides as a treatment. Since *Bt*-crops as a pest control measure are intended to replace or reduce applications of conventional insecticides commonly used in agriculture, they should be considered as baseline for a comparative risk assessment (Dale *et al.* 2002, Conner *et al.* 2003). Experiments that included broad spectrum insecticides, such as pyrethroids and organophosphates, have shown consistently reduced abundances of different groups of predators and hymenopteran parasitoids (*Bt*-maize: Musser & Shelton 2003, Candolfi *et al.* 2004, Meissle & Lang 2005; *Bt*-cotton: Wu & Guo 2003, Bambawale *et al.* 2004, Men *et al.* 2004, Hagerty *et al.* 2005, Naranjo 2005a, Whitehouse *et al.* 2005). Side effects of more selective insecticides such as indoxacarb (oxadiazine) or spinosad (macrolide) largely depended on the spray frequency (Musser & Shelton 2003) whereas systemic insecticides (such as imidacloprid, a neonicotinoid) were found to have no or little effect on natural enemies (de la Poza *et al.* 2005). Although some of the field studies were limited in their spatial scale, and they were lacking statistical power due to limited replication and high variability in the data, they clearly indicated that non-target effects of *Bt*-crops were substantially lower than those of broad spectrum insecticides. This has been confirmed by recent large-scale studies conducted in commercially managed *Bt*- and non-*Bt*-cotton fields in the United States (Head *et al.* 2005, Torres & Ruberson 2005).

Surprisingly few studies have compared the biological control functions of natural enemies in *Bt*- and conventional crops (Romeis *et al.* 2006). Parasitization rates of naturally occurring or sentinel larvae of sensitive (target) lepidopteran species in *Bt*-crops have often been reported to be lower compared to control plots (Johnson 1997, Siegfried *et al.* 2001, Bourguet *et al.* 2002, Manachini 2003). This reduction in parasitism is not surprising given that host populations were significantly decreased by the *Bt*-crop. Predation rates on sentinel lepidopteran eggs or larvae were measured in *Bt*-sweet corn and in *Bt*-cotton. They did not differ between *Bt*- and untreated non-*Bt*-fields (Reed *et al.* 2001, Musser & Shelton 2003, Naranjo 2005b), but were significantly reduced by the application of broad-spectrum insecticides (Musser & Shelton 2003). Studies in commercially managed cotton fields revealed much higher predation rates in *Bt*-cotton compared to non-*Bt*-fields where more insecticides were applied (Head *et al.* 2005).

6.3.2 Effects on pollinators
Many insect species are known to act as pollinators of various crops and wild plants. They are therefore of great ecological and economic importance. Among the various insect pollinators, honey bees are the best known, but it is now recognized that other species like bumble bees and solitary bees are also important in ensuring pollination of many plant species.

Pollen feeding represents the most likely route of exposure to *Bt*-toxins for adult honey bees (Malone & Pham-Delegue 2001), because pollen is the main source of proteins for adult bees (Crailsheim 1990). Honey bees collect pollen from a wide range of plants during a foraging season, but interestingly, only five plant species, including maize,

account for more than half of the pollen collected in Switzerland. Especially in summer, when other pollen sources are scarce, maize pollen can account for a substantial amount of the collected pollen (Wille *et al.* 1985). Although adult bees could be exposed to transgenic maize pollen, the potential hazard of *Bt*-pollen depends on the amount of toxin expressed in the pollen. For the most widely commercially used *Bt*-maize events MON810 and Bt11 this amount (< 0.09 μg / g pollen) is almost 80-times lower in the pollen than in Event 176 (7.1 μg / g pollen) (Stanley-Horn *et al.* 2001), an event that has meanwhile been withdrawn from the market. A case-specific risk assessment estimating the hazard from *Bt*-pollen exposure has to be performed for every GM maize-event individually, since other events such as MON863 (*Bt*-maize expressing the beetle-specific Cry3Bb) may express higher levels of *Bt*-toxin in the pollen. With regard to honey bees, the larval stages are far less exposed to transgenic products than adults, because pollen constitutes only a minor part of the protein supply of honey bee larvae, contributing less than 5% to the total amount of protein necessary for larval development (Babendreier *et al.* 2004). Larvae of bumble bees and solitary bees, in contrast, rely on large amounts of pollen to complete development. However, both groups of bees have not been observed collecting maize pollen.

Because of their ecological and economic importance, honey bees are often used as test species in pre-market risk assessment studies to assess direct toxicity on non-target organisms. Such studies have been conducted for each *Bt*-crop prior to its registration in the United States (EPA 2001). Feeding tests with Cry1Ab proteins were conducted on both honey bee larvae and adults and in each case no effects were observed (EPA 2001). Further studies with bees fed with purified *Bt*-proteins and with pollen from *Bt*-crops, as well as when bees were allowed to forage on *Bt*-crops in the field have confirmed the lack of effects noted by the U.S. EPA (Malone & Pham-Delegue 2001, Malone 2004, Babendreier *et al.* 2005, O'Callaghan *et al.* 2005).

6.3.3 Effects on butterflies
Butterflies are considered species with a high aesthetic value serving as symbols for conservation awareness. Since Cry1Ab is selectively toxic to Lepidoptera (moths and butterflies), off-site pollen flow from *Bt*-maize fields might potentially have adverse effects on Lepidopteran species if their larvae feed on host plants dusted with *Bt*-pollen. The case of *Bt*-maize pollen and the Monarch butterfly (*Danaus plexippus*) (Losey *et al.* 1999) caused much public interest and led to a debate over the potential risks and the environmental impact of *Bt*-maize. However, extensive studies showed that risks for the monarch butterfly were negligible, and that reports of toxicity of high doses of Cry1Ab toxin to monarch butterflies in the laboratory did not necessarily mean that there would be exposure to toxic levels in the field (see Box 4 for details). At the time of the conditional registration of *Bt*-maize Event 176 in 1995, the potential hazard of maize pollen containing Cry1Ab toxin on threatened or endangered Lepidopteran species had been identified (NRC 2002). An examination of an overlay map showing the distribution of endangered lepidopteran species and maize production areas revealed that listed lepidopteran species do not occur in agricultural areas where maize is grown. Furthermore maize is not considered a host plant for these species (EPA 2001). The map also indicated that potential concerns were restricted to the Karner blue butterfly (*Lycaeides melissa samuelis*). After having received and obtained additional data, EPA conducted a second ecological risk assessment for potential impacts to the Karner blue (EPA 2001). They estimated the toxicity of pollen from the currently registered Cry1Ab *Bt*-maize products to Karner blue larvae being very low. Furthermore the exposure of larvae to *Bt*-maize pollen is limited because maize and lupine (the host plant serving as food source for Karner blue

Box 4: The case of the Monarch butterfly

Losey and his colleagues (1999) found that when pollen from a commercial variety of *Bt*-maize (event Bt11) was spread on milkweed leaves in the laboratory and fed to monarch butterfly larvae, the larvae consumed significantly less from these leaves compared with leaves dusted with non-transgenic pollen. In addition, after four days almost half of the tested larvae died, which was significantly more than on the leaves with non-transgenic pollen where none of the tested larvae died. In a similar study, Jesse and Obrycki (2000) used *Bt*-maize pollen (Event 176) covered leaf samples of milkweed taken from within and at the edge of maize fields and fed them under laboratory conditions to first instar monarch butterfly larvae. Within 48 hours, mortality in larvae feeding on *Bt*-pollen was found to be significantly higher, compared to non-*Bt*-pollen and a no pollen control. The results of the studies drew a lot of attention to (potential) effects of *Bt*-crops on butterflies since the Monarch is considered a "conservation flagship species" in the United States. However, both studies also received a lot of criticism and scientist questioned the validity of conclusions based on the data obtained in laboratory studies. The critics felt that, in addition to the mere toxicity (hazard), an ecological risk assessment has to consider whether and how the monarch will encounter the *Bt*-toxin (exposure). They also felt that the studies most likely did not address questions like the spatial and temporal overlap of monarch larvae and *Bt*-pollen. Subsequent studies therefore determined where the monarchs occur during their breeding season (Oberhauser *et al.* 2001), and what percentage of the population of monarchs is possibly affected be the *Bt*-toxin in areas where *Bt*-maize is presently grown (Sears *et al.* 2001). The results showed that larval exposure to pollen on a population-wide basis is low, given the proportion of larvae in maize fields during pollen shed, the proportion of *Bt*-maize fields, and the levels of pollen within and around maize fields (Oberhauser *et al.* 2001). The proportion of monarch butterfly population exposed to *Bt*-pollen was estimated to be less than 0.8% (Sears *et al.* 2001).

Another severe point of criticism concerned the amount of pollen used in the feeding experiments. Losey *et al.* (1999) made no attempt to quantify the pollen dose on the leaves or to determine whether these levels used in the laboratory assay reflected realistic levels, which the larvae would be exposed in the field. In fact, subsequent studies revealed that larvae were exposed to less quantities of maize pollen on the host plant than had been thought before. Pollen density on the upper leaves, where the monarch egg masses are laid, was only 30–35% of that on middle leaves, and pollen densities were significantly higher around the leaf midrib, an area avoided by younger larvae (Pleasants *et al.* 2001). Laboratory bioassays also showed that the only transgenic *Bt*-maize pollen that consistently affected monarch larvae was from Event 176, an event that has meanwhile been withdrawn from the market. Pollen from the most widely planted *Bt*-maize events (MON810 and Bt11) showed to have no acute effects on larvae in field settings (Hellmich *et al.* 2001, Stanley-Horn *et al.* 2001) since their pollen expresses 80-times less toxin than Event 176 (Stanley-Horn *et al.* 2001). The results suggest that pollen densities used by Losey *et al.* were in excess compared to pollen densities present in maize fields or that the pollen of event Bt11 used by Losey *et al.* may have been contaminated with non-pollen tissues (Anderson *et al.* 2004). Excessive pollen densities of the currently commercialized events (Bt11 and MON810) would be required to obtain relevant adverse effects on larval developments (Hellmich *et al.* 2001). These excessive pollen densities, however, would most probably prevent Monarchs from ovipositioning on these leaves (Tschenn *et al.* 2001).

In conclusion, continuous exposure of monarch butterfly larvae to natural deposits of *Bt*-pollen on milkweed plants within maize fields can affect individual larvae (Sears *et al.* 2001, Dively *et al.* 2004). However, long-term exposure of monarch butterfly larvae throughout their development to *Bt*-maize pollen is detrimental to only a fraction of the breeding population because the risk of exposure is low. It is unlikely that *Bt*-maize will affect the sustainability of monarch butterfly populations in North America (Sears *et al.* 2001, Dively *et al.* 2004). Effects of *Bt*-maize should be compared to mortality due to other factors, which is very high in natural monarch butterfly populations, and averages around 80% over the entire larval development period (Oberhauser *et al.* 2001, Dively *et al.* 2004). More important factors that may influence monarch butterfly survival include loss of over wintering habitats in Mexico, use of insecticides to control lepidopteran pests, and accidents such as collision with automobiles (EPA 2001).

larvae) do not generally overlap. Wild lupine does not occur at all in maize fields and it is not expected to grow adjacent to maize fields. Notably, the evaluations concentrated only on endangered and threatened species, and Monarch butterflies were not considered being either of both. The risk to this species was therefore not considered in the risk analyses conducted by the U.S. regulatory agencies (NRC 2002).

Considering the substantial public interest the case of the Monarch butterfly caused in Europe, surprisingly few studies have been published on potential risks of Cry1Ab on European butterflies. Although a number of studies were launched in different European countries, most of the studies published were mere hazard studies conducted in the laboratory (Felke & Langenbruch 2001, Felke *et al.* 2002, Felke & Langenbruch 2003). In a theoretical exposure assessment, Schmitz *et al.* (2003) estimated that approximately 7% of the relevant German butterfly species occur in farmland areas and could potentially be affected by *Bt*-pollen exposure. Of these species, however, only 14% (= 1% of the total) were found to be potentially exposed on a regional scale.

6.3.4 Effects on herbivorous insects

Effects of *Bt*-maize on non-target herbivorous insects have been assessed less frequently than those on natural enemies. This could be due to the fact that most herbivores occurring in crops are considered potential crop pests, possibly causing yield losses. Cry1Ab has shown to be effective against various other lepidopteran species that are not the primary target (Pilcher *et al.* 1997, Gonzales-Nunez *et al.* 2000, Dutton *et al.* 2005). In many cases this is regarded as being a positive effect, since additional crop protection measures can be reduced, especially where pest control measures are difficult to perform. Some groups of herbivores, including Thysanoptera (thrips) (Obrist *et al.* 2005) and Tetranychidae (spider mites) (Dutton *et al.* 2002, Obrist *et al.* 2006) ingest the *Bt*-toxin when feeding on *Bt*-crops. However so far, no effects have been detected in these groups of herbivores. Furthermore, several studies have demonstrated that aphids do not take up *Bt*-toxins, since these are not present in the phloem sap, and therefore neither aphids nor the predators feeding on them are likely to be exposed to the *Bt*-toxin (Head *et al.* 2001, Raps *et al.* 2001, Dutton *et al.* 2002). In some studies, herbivore abundance was assessed in field studies, however, these surveys were primarily conducted to determine non-target effects on natural enemies (Bourguet *et al.* 2002, Candolfi *et al.* 2004, Lumbierres *et al.* 2004, Pons *et al.* 2005, Eizaguirre *et al.* 2006). The results showed that the number of individuals of different species varied greatly from year to year and between locations, but no consistent tendencies related to *Bt*-maize were recorded. One study found a higher density of aphids on *Bt*-maize, but it was concluded that the expression of the *Bt*-toxin could not be the cause of this effect (Lumbierres *et al.* 2004). The authors estimated that factors affecting the process of aphid settlement or retention on plants, such as host attraction or plant structure, could be a possible cause for this observation. The results further suggest that economic implications such as increased insecticide applications are not to be expected, because the higher density of aphids in *Bt*-maize does not continue throughout the season (Lumbierres *et al.* 2004).

6.3.5 Changes from secondary pests to primary pests

Secondary pest outbreaks generally occur when pesticide applications kill natural enemies that were controlling a species that was not a pest before. These species can increase to densities that cause damage, because the natural enemies previously maintaining their populations at low densities are no longer present or abundant enough to control them (Hajek 2004). The adoption of *Bt*-crops (especially *Bt*-cotton) has resulted in considerable reductions in the use of broad-spectrum insecticides (Fitt *et al.* 2004). The

occasionally observed increase of some herbivore groups (mirids and stinkbugs) in some *Bt*-cotton-growing regions (Riddick *et al.* 1998, Greene *et al.* 2001, Wu *et al.* 2002) could, however, not be attributed to a disturbance of biological control functions. These changes were more likely due to the replacement of broad-spectrum insecticides (that were previously controlling these herbivores) by *Bt*-crops having a very specific mode of action and targeting only a narrow group of species (Greene *et al.* 2001). As yet, there is little evidence in any country that secondary pest outbreaks have emerged in *Bt*-crops as a problem requiring substantial use of additional insecticides (Whitehouse *et al.* 2005). It even seems that the overall biological control functions are positively influenced by the use of *Bt*-crops (Fitt *et al.* 2004, Naranjo 2005b, Whitehouse *et al.* 2005) since observed decreases in some pest groups such as aphids were linked to higher abundances of natural enemies due to the absence of insecticides (Reed *et al.* 2001, Wu & Guo 2003).

6.4 Conclusions and interpretation of non-target study results

The results of the various studies that have been performed during the last years provide evidence that *Bt*-maize and *Bt*-cotton expressing insecticidal Cry1-proteins are more specific and have fewer side effects on non-target arthropods than most insecticides currently used. Except for the lepidopteran species the toxin is intended for, Cry1Ab does not cause direct toxic effects on any of the arthropods groups examined. Indirect toxic effects of *Bt*-maize on natural enemies caused by changes in the availability and/or the quality of target herbivores as prey and host items may occur. Specialist predators and parasitoids are likely to be most sensitive to changes in host/prey quality or quantity if their survival depends upon the supply of pest insects. Such indirect toxic effects are, however, foreseeable because the reduction of pests is the obvious goal of any crop protection method. (O'Callaghan *et al.* 2005, Romeis *et al.* 2006). Many natural enemies are polyphagous and in the field they are able to switch to other preys when one particular food source is scarce. It is therefore not clear to what extent indirect prey-mediated effect will be relevant in the field. Furthermore, the occurrence of indirect toxic effects is not restricted to GM technology, as any pest control measure will cause a reduction in prey numbers, which will consequently affect population densities of natural enemies (Clark *et al.* 2005, O'Callaghan *et al.* 2005, Romeis *et al.* 2006).

Summary: Effects of GM crops on non-target organisms

- The results of the various experimental field studies that have been performed during the last years provide evidence that *Bt*-maize expressing the insecticidal protein Cry1Ab is more specific and has fewer side effects on non-target arthropods when compared to currently used insecticides.

- No adverse effects on non-target natural enemies resulting from direct toxicity of *Bt*-crops have so far been observed in the field. Experimental field studies have only revealed minor transient or inconsistent effects of *Bt*-crops when compared to a non-*Bt*-control.

- Indirect prey-quality mediated effects due to *Bt*-maize may occur, but they can be considered being subtle shifts in the arthropod community caused by the effective control of the target pest.

- Extensive studies showed that risks from *Bt*-maize for the monarch butterfly were negligible, and that reports of toxicity of high doses of Cry1Ab protein to monarch butterflies in the laboratory did not necessarily mean that there would be a risk for monarch butterfly populations in the field.

7 Impacts of GM crops on soil ecosystems

Soil is responsible for irreplaceable ecosystem services such as sustaining terrestrial primary production including the support of crop production. Key functions of the soil include i) biomass production, ii) regulation of water quantity and quality, iii) regulation of element cycling (e.g. nutrients), iv) mechanical support for living organisms, v) habitat function, vi) carbon balance (including sequestration), and vii) biodegradation of waste (Cartwright *et al.* 2004). Although a common definition for "soil quality" itself has not been found yet, the capacity to provide and sustain these functions is often described as soil quality. Typically, chemical, physical and biological indicators are used to determine soil quality. The evaluation is, however, complex because all three indicator groups are subject to high natural variability both in time and space, making an overall interpretation of scientific studies difficult.

Modern agricultural systems influence chemical (e.g. use of persistent pesticides), physical (e.g. erosion and compaction by machinery) and biological (e.g. symbiotic N_2-fixing bacteria) parameters of the soil. Key influences of plants on soil ecosystems occur via root exudates, the deposition of plant litter, as well as water, gas and nutrient exchange (Cartwright *et al.* 2004). These interactions largely influence soil organisms (e.g. by the supply of carbon), most of all the microbial community. In return, modifications of the microbial community affect higher trophic levels and modify soil processes such as nitrogen cycling, which influences the capacity of soils to sustain primary production.

The proximity and dependency of interactions between plants and soil ecosystems indicate that, similar to any agricultural crop, GM crops will influence soil processes. The main questions are whether the commercial cultivation of GM crops is inducing changes in biodiversity and soil processes and whether these changes exceed natural variation caused by a multitude of environmental factors or the variation found in conventional crop systems caused by different crops, cultivars, crop rotation as well as the impacts of agricultural operations such as tillage. It is important to evaluate the ecological significance of changes induced by GM crops in the context of these variations and to assess whether changes, such as the accumulation of toxins or altered community structure of soil organisms, prove to be reversible.

The following chapter discusses the concern that non-target soil organisms and processes could be affected by the accumulation of *Bt*-toxins in soils through cultivation of the currently commercialized *Bt*-crops. Impacts of altered weed management practices accompanying the cultivation of GMHT crops are discussed in chapter 10.

7.1 Release of *Bt*-toxins into soil

Because *Bacillus thuringiensis* (*Bt*) is a soil bacterium, *Bt*-toxins are naturally occurring in soils. In addition, *Bt*-spray formulations are commonly used for insect control in agriculture and forestry (Walker *et al.* 2003). Due to their high specificity they are regarded as environmentally friendly and have rarely been found to have adverse effects on non-target organisms and human health (Glare & O'Callaghan 2000). Despite the history of safe use of *Bt*-spray formulations it appears that concerns related to the dispersal of *Bt*-toxins in the environment are perceived more strongly and are judged more important since the adoption of GM crops.

Bt-toxins expressed in *Bt*-crops can enter the soil system via root exudates, senescent plant material remaining on or in the ground after harvest, as well as damaged and cast-off dead root cells (Saxena *et al.* 1999, Zwahlen *et al.* 2003a, Baumgarte & Tebbe 2005). In addition to the amount of crop debris after harvest determining the amount of *Bt*-toxin added to the soil system, the protein quantity also depends on the level of expression, which can differ both between tissue and crop. In a comparative study, *Bt*-maize, -potato and -rice all contributed to *Bt*-toxins in the soil via root exudation, whereas *Bt*-cotton, -oilseed rape and -tobacco did not (Saxena *et al.* 2004). When comparing 12 different *Bt*-maize hybrids expressing Cry1Ab which represented three transformation events (Bt11, MON810 and Event 176), no differences in exudation levels were measured (Saxena *et al.* 2002). In a three year field study with *Bt*-maize event MON810, the supply of toxins by root exudates continued throughout the season, and *Bt*-levels in the soil did not correlate with a specific plant growth period (Nguyen Thu 2004, Baumgarte & Tebbe 2005). As to be expected, the continuous supply via root exudates lead to higher toxin concentrations in the rhizosphere compared to the surrounding bulk soil.

The supply of *Bt*-toxins by senescent plant material mainly occurs via decaying biomass remaining on or in the ground after harvest. The toxin input from senescent plant tissue varies, depending on initial expression levels, the progression of decay of the plant cells and the biomass remaining in the field. Expression levels in leaves in the *Bt*-maize variety MON810, for example, are estimated to be around 4–7 times higher than in the roots (Mendelsohn *et al.* 2003). However, when comparing plant material in the field after harvest, leaf material only contained 0.2% of the initial toxin concentration, whereas root material still contained 12% (Baumgarte & Tebbe 2005). The difference between remaining toxin concentrations in roots compared to leaves was explained by a potentially higher turnover rate of leaf material compared to roots. It was therefore concluded that *Bt*-maize roots can be a major reservoir of *Bt*-toxins in soil.

7.2 Persistence and biological activity of *Bt*-toxins in soil

Persistence of *Bt*-toxins in soil is primarily depending on the protein quantity added (see chapter 7.1) and on the rate of inactivation and degradation by biotic and abiotic factors (Dubelman et al. 2005). Degradation rates of *Bt*-toxins are known to be influenced by soil type, environmental conditions, the protein source (purified versus plant-produced) as well as the particular Cry-protein chosen (Clark et al. 2005). Persistence in the environment can be expressed in different ways, which affects comparison between studies. Terms such as dissipation time to 50% (DT50) or half-life are used to describe the time until 50% of the original amount of a substance is degraded. Persistence can also be described in terms of detectable residues. While for example a DT50 of 1–2 days is an indicator for a rapid rate of dissipation, detectable residues after 2–6 months indicate that some small amounts of the protein last in a biologically active form (if detected by a bioassay) or in an immunologically active form (if detected by ELISA). Description of detectable residues is a reference to an amount of substance that can be determined by an analytical method, but is not necessarily indicating biological activity. Determination of biological activity requires the use of a sensitive organism to indicate toxic activity (Clark et al. 2005).

Persistence, degradation and inactivation of *Bt*-toxins have been assessed in a number of studies (Tab. 3). The majority of the studies were conducted with *Bt*-maize expressing Cry1Ab including three studies assessing persistence and biological activity in soil during

Table 3. Summary of results from selected studies assessing persistence, degradation and inactivation of *Bt*-toxins in soil

Study conditions	Toxin incorporation into soil	*Bt*-crop *Bt*-toxin	*Bt*-toxin detection	Persistence (days)	Refe-rence
Lab	Experiments were carried out with field grown cotton tissue / soil / purified toxins in microcosms	Cotton tissue Cry1Ab and Cry1Ac	Detectable residues (ELISA) [a]	Detection of toxin and insecticidal activity at termination of test – 28 d (Cry1Ab) and 56 d (Cry1Ac)	1)
Lab	Purified toxin and transgenic leaves added to soil in microcosms. Toxins extracted and measured for 140 days	Microbial toxin and cotton tissue Cry1Ab and Cry1Ac	Detectable residues (ELISA)	Initial rapid degradation, low percentage may persist for weeks/months. Half lives at 22/40d, depending on clay/organic content of soil	2)
Lab / Greenhouse 24–27°C	GM plants grown in greenhouse, harvest 2 weeks after pollen shed. Maize tissue was incubated with and without soil and mixed into artificial insect diet. Dose-weight response determined bioactivity. Soil: high clay content (25%)	Maize tissue Cry1Ab	Bioactivity test [b]	1.6 d (in soil) DT50 [c] 15.0 d (in soil) DT90 25.6 d (no soil) DT50 40.7 d (no soil) DT90	3)
Lab / Field Autumn /winter MO, USA	Protein incubation in soil for 120 d. Bioassay based on growth inhibition to determine DT50	Cotton tissue Cry2A	Bioactivity test	15.5 d (lab) DT50 31.7 d (field) DT50 120 d: down to <25% (field&lab)	4)
Lab and field Includes period of frost	Rhizosphere soil sampled from *Bt*-maize in a plant growth room and in the field	Maize tissue Cry1Ab	Western blot Bioactivity test	180d: *Bt*-toxin detectable in rhizosphere soil samples from field (after first frost) around plants that had been dead for several months	5)
Lab 25°C	Mixture of Cry1F pipetted onto soil samples representative of cotton fields	Microbial toxin, Cry1F	Bioactivity test	< 1 d DT50	6)
Field ~16°C	Soil samples were collected 3 months after post harvest tillage for 3-6 consecutive years	*Bt*-cotton cultivation Cry1Ac	ELISA Bioactivity test	No detectable *Bt*-toxins in any of the samples	7)
Litter bags in field (CH) ~9°C Soil cages in field (CH) ~9°C	Leaves (growth chamber) sampled before/after pollen shed, cut&dried and placed in litter bags (5mm mesh) and buried in soil in mid-October. Monthly analysis. Leaves sampled 3 weeks after pollen shed, cut&dried and added to surface of soil cages (1mm mesh) with earthworm, tied up in field for 200 d, starting December	Maize tissue Cry1Ab	ELISA Bioactivity test	45 d DT50 145 d DT90 240 d: <1.5% No degradation in winter (<5°C) 35 d DT50 105 d DT90 200 d: 0.3% Degradation continued in winter	8)
Lab and field No temperature indication	Lab: *Bt*-maize residues added to soil and incubated for 43 days. Field: soil samples from experimental fields after 4 years cultivation of *Bt*-maize	Maize tissue Cry1Ab	ELISA	Lab: 14 d : Cry1Ab not detectable Field: most *Bt*-toxin in subsurface soil at 0–15cm depth. Not clear if *Bt*-toxin from previous year	9)
Field No temperature indication MO, USA	After ≥ 3 years commercial cultivation of *Bt*-maize, soil samples were collected during growth period and 6 weeks after harvest. Growth inhibition determined presence of toxin	Maize tissue Cry1Ab	Bioactivity test	No evidence of persistence or accumulation	10)
Field No temperature indication Germany	Samples were taken during a 3-year monoculture study with MON810 from bulk and rhizosphere soil at a) 9 leaves per plant, b) stem elongation phase, c) flowering/anthesis, d) ripening	Maize tissue Cry1Ab	ELISA	No accumulation during growing season despite potential binding to soil particles. Proportion of toxin persisted through winter but no indication of accumulation, toxin in rhizosphere remained consistently higher than in bulk soil	11)

1) Donegan *et al.* (1995); 2) Palm *et al.* (1996); 3) Sims & Holden (1996); 4) Sims & Ream (1997); 5) Saxena & Stotzky (2000); 6) Herman *et al.* (2001); 7) Head *et al.* (2002); 8) Zwahlen *et al.* (2003a); 9) Hopkins & Gregorich (2003); 10) Dubelman *et al.* (2005); 11) Baumgarte & Tebbe (2005)

a) ELISA: Enzyme-Linked Immunosorbent Assay; b) Bioactivity test: sensitive insect bioassay
c) DT50: Dissipation time 50% = time required for one-half of the initial quantity or concentration to dissipate from a system

commercial *Bt*-maize cultivation (Saxena & Stotzky 2000, Hopkins & Gregorich 2003, Dubelman *et al.* 2005). The other studies were either performed with *Bt*-cotton containing other Cry proteins or with purified toxins. When comparing the results on persistence, degradation and inactivation of *Bt*-toxins in soil, it has to be considered that many of the influencing factors such as temperature, soil type, study parameters and experimental design differed between the various studies. Estimation of DT50 range from a few hours (Herman *et al.* 2001) to 32 days (Sims & Ream 1997) (Tab. 3). Apart from Sims & Ream (1997), most of the laboratory studies indicate instability of the toxin and rapid initial degradation. Results of field studies evaluating persistence and biological activity range from "no evidence of toxins in soil after commercial cultivation of *Bt*-crops" (Head *et al.* 2002, Dubelman *et al.* 2005) to "detectable residues of *Bt*-toxins at the end of field experiment" (Zwahlen *et al.* 2003a). A relatively long period of persistence was measured in two semi-field experiments by Zwahlen *et al.* (2003a). Initially, 80% and 62% degradation took place within 60 and 40 days, respectively, and by the end of the experiment, 240 respectively 200 days later, less than 1.5% of the initial *Bt*-toxin content could be detected. The experiments were carried out in the field starting late-autumn/winter using buried litter bags as well as soil cages containing plant material on the soil surface. In contrast to other studies, which were typically carried out at temperatures of 24–28°C, the study was performed at temperatures averaging 9°C including a longer period during winter time with presumably no degradation activity.

Because temperature is an important factor influencing degradation, an increase of 10°C in soil temperature leads to a two to three fold level of microbial activity and can therefore lead to higher rates of decomposition (Pont & Nentwig 2005). Similar to the relatively long persistence described by Zwahlen *et al.* (2003a), insecticidal activity of *Bt*-toxin residues was detectable in the soil for several months after the first frost, which was likely to be due to low temperature (Saxena & Stotzky 2000). Evaluations of insecticidal activity and persistence of Cry1Ab after commercial cultivation of *Bt*-maize, however, were not able to detect any residual *Bt*-toxin (Hopkins & Gregorich 2003, Dubelman *et al.* 2005). Repeated cultivation of Cry1Ab-expressing maize plants for three or more consecutive growing seasons did not result in accumulation or persistence of Cry1Ab protein in soil (Dubelman *et al.* 2005). Similarly, no Cry1Ac protein was detected using both ELISA and a bioassay in soil after multiple years of transgenic *Bt*-cotton cultivation (Head *et al.* 2002). The results indicate that there is no evidence for insecticidal activity or accumulation of *Bt*-toxins in the soil under commercial cultivation conditions.

Some of the variation in decomposition rates found by Zwahlen *et al.* (2003a) and Head *et al.* (2002) could be explained by differences between crop species in the relative C:N ratio, which are thought to affect decomposition rates resulting in a two to three times faster degradation in cotton than in maize. Variation could further be explained by the binding of *Bt*-toxins to surface-active particles. A series of studies document that *Bt*-toxins readily bind to clay particles and humic acid components in soil (Venkateswerlu & Stotzky 1992, Tapp & Stotzky 1998, Saxena *et al.* 1999, Saxena & Stotzky 2000, Stotzky 2004). Binding reduces the bioavailability of the protein to microorganisms and in consequence reduces the degradation of the *Bt*-toxin. The results of bioassays suggest that bound *Bt*-toxins retain their insecticidal activity. As a consequence, persistence and biological activity of *Bt*-toxins may be different depending on the respective soil type.

In conclusion, the presented studies generally indicate an exponential degradation of *Bt*-toxins. After a short lag phase due to the breakdown of plant cells, a rapid degradation of the *Bt*-toxins takes place with low amounts that may persist in soil after one

season. *Bt*-toxins may partially persist as a consequence of their binding to surface-active clay particles and it seems that bound proteins retain their insecticidal activity. To date, none of the laboratory or field studies suggest accumulation of *Bt*-toxins in soil over several years of cultivation. Experience from commercial cultivation indicates that *Bt*-toxin will not persist for long periods under natural conditions. Although estimates on persistence of *Bt*-toxins differ among studies ranging from hours to months, the results are not essentially conflicting. Much of the described variation can be explained by the fact that the studies employed various parameters and experimental designs. In addition to environmental conditions varying between sites and seasons, degradation and persistence were depending on a multitude of factors including the type of *Bt*-toxin (e.g. Cry1Ab), the crop species (e.g. differences in C:N ratio), biotic activity (e.g. temperature), soil type (e.g. clay content), and the applied crop management practices (e.g. no-till with roots remaining in the soil).

7.3 Impacts on soil organisms

Microorganisms are the dominant organisms both in terms of biomass and activity in the soil (Bruinsma *et al.* 2003). The soil microfauna is involved in a number of important processes including decomposition of organic matter, nutrient mineralization, regulation of plant pathogens, decomposition of agricultural chemicals, and improvement of soil structure (Gupta & Yeates 1997). The close interaction between crop cultivation and soil processes inadvertently leads to contacts of soil organisms with *Bt*-toxins released from GM crops. The here discussed data on the influence of *Bt*-crops on soil organisms originates from studies performed under laboratory and field experimental conditions, however, none of the data originates from commercial *Bt*-crop cultivation.

7.3.1 Bacteria and fungi

To date, effects of *Bt*-crops on microorganisms have been evaluated in a number of studies, which have used a range of different parameters and techniques (Bruinsma *et al.* 2003). No consistent significant differences in bacterial counts were detected in a greenhouse study comparing *Bt*- and non-*Bt*-maize (Brusetti *et al.* 2004). As expected major differences were present in bacterial communities in the bulk soil compared to the rhizosphere, differences which were, however, unrelated to the specific cultivar. In this study, differences were only detectable when using molecular profiling techniques, whereas conventional culturing techniques did not reveal any differences (Brusetti *et al.* 2004). In a study using cultured bacteria and fungi incubated with soil samples containing Cry1Ab root exudates and decomposing plant tissue, no significant influence of the *Bt*-toxin was detected (Saxena & Stotzky 2001a). A comparison performed in a growth chamber found that microbial community structure was mostly determined by the soil type (Blackwood & Buyer 2004). In a field study with MON810, bacterial community structure seemed to be less affected by the Cry1Ab protein than by age of the plants and field heterogeneity (Baumgarte & Tebbe 2005). A further field study found that different maize cultivars induced greater differences than the cultivation of *Bt*-maize versus non-*Bt*-maize (Griffiths *et al.* 2005).

Using an experimental model system to monitor impacts of *Bt*-maize on arbuscular mycorrhizal fungi, root exudates of Event 176 maize were reported to affect the life cycle of the fungus *Glomus mosseae* by reducing pre-symbiotic hyphal growth, which was not the case with Bt11 or control plants (Turrini *et al.* 2004). The authors stated that their findings, which coincided with the findings of a microcosm and greenhouse study showing significantly lower levels of mycorrhizal root colonization in Event 176 (Castaldini *et*

al. 2005), could possibly be explained by expression levels of Cry1Ab toxin being considerably lower in Bt11 compared to Event 176 (Turrini *et al.* 2004). This interpretation, however, is questionable since Turrini *et al.* (2004) have not directly measured Cry1Ab expression levels in roots, but were referring to expression levels of Cry1Ab that are found in pollen based on data provided by the U.S. EPA (EPA 2001). The probability that the found effects on arbuscular mycorrhizal fungi have been caused by direct exposure to *Bt*-toxins released from roots of Event 176 can be expected to be relatively low considering that this event does not express Cry1Ab toxin in roots (Koziel *et al.* 1993, Fearing *et al.* 1997, Dutton *et al.* 2003, Nguyen Thu 2004).

In conclusion, most studies detected some differences when comparing *Bt*- with non-*Bt*-maize, however, the use of a wide variety of techniques makes a comparison among studies difficult (Bruinsma *et al.* 2003). The reasons for the observed differences as well as their implications are usually not clear. One difficulty in evaluating these changes is the high number of species of microbial soil communities and the natural variability occurring therein. In addition, the (species and functional) diversity of microbial soil communities is influenced by a multitude of environmental factors including plant species, water stress, fertilisation, field management, tillage, fungal disease, grassland improvement, nitrification and soil depth (Cartwright *et al.* 2004). Knowledge on the complex diversity of soil microorganisms is limited since only a small portion of soil microbial populations can be cultured and identified using standard analytical methods (Motavalli *et al.* 2004). Due to this limited knowledge, the importance and the functional consequences of detected differences in soil microbial populations are difficult to determine. Some methodological approaches, including the use of molecular biological techniques, show some promise in helping to understand the impact of GM crops on soil microbial ecology (Bruinsma *et al.* 2003). These molecular techniques yield fingerprint-type data, which represents an image of the soil microbial community analyzed. An accepted definition of the taxonomic unit, which can be used for defining soil microbial diversity, is, however, clearly lacking (Widmer & Oberholzer 2003). Because most studies assessing effects of GM crops on soil ecosystems have not determined the natural variation occurring in agricultural systems, it is generally difficult to establish whether the differences between *Bt*- and non-*Bt*-crops were exceeding this variation. The only study considering natural variation suggests that observed differences between *Bt*- and non-*Bt*-crops were not as large as differences caused by environmental parameters or by agricultural practices (Griffiths *et al.* 2005).

7.3.2 Nematodes

Impacts of Cry1Ab toxins on nematodes were examined in three studies using soil samples from fields planted with *Bt*-maize and the non-*Bt*-isoline (Saxena & Stotzky 2001a, Manachini & Lozzia 2002, Griffiths *et al.* 2005). Results of a study by Saxena & Stotzky (2001a) indicated that there were no significant differences in the number of nematodes between rhizosphere soil of *Bt*- and non-*Bt*-maize. In a field study using Event 176, no overall significant influence on communities and biodiversity of nematodes were found when comparing *Bt*- and non-*Bt*-samples (Manachini & Lozzia 2002). In one of the eight study regions, however, fungi feeding nematodes were found to be more abundant in the *Bt*-maize field, while bacteria-feeding nematodes were more abundant in the field cultivated with the isogenic hybrid (Manachini & Lozzia 2002). In field studies conducted in the EU-funded ECOGEN project covering different soil types, distinct climate zones as well as cropping years, MON810, the near isogenic non-*Bt*-cultivar, a further maize cultivar and plots of grass were evaluated (Griffiths *et al.* 2005). In all sites, nematode numbers associated with *Bt*-maize were reduced. Since reduced nematode numbers

were not limited to a particular site or trophic group these results could indicate a direct influence of *Bt*-toxins rather than an indirect food-related effect. The reason for this reduction is probably due to a combination of factors and is not further elucidated by Griffiths *et al.* (2005). The analysis of the more relevant nematode community structure, however, revealed distinctly different communities in each experimental site and found no relation to *Bt*- or non-*Bt*-cultivation. The differences caused by the cultivation of *Bt*-maize were not as large as those resulting from cultivating different conventional maize cultivars, different crop plants, or as large as the differences between sites or sampling dates. It was concluded that the effects found in *Bt*-maize fall within the normal variation expected in agricultural systems (Griffiths *et al.* 2005). Although smaller nematode population numbers were sometimes found under *Bt*-maize, overall, all studies indicate no consistent significant effects on nematode community structure.

7.3.3 Woodlice

Three laboratory studies have shown that *Bt*-maize expressing Cry1Ab has no deleterious effects on the woodlice *Porcellio scaber* (Escher *et al.* 2000, Wandeler *et al.* 2002, Pont & Nentwig 2005). Feeding experiments with *P. scaber* showed that consumption rates did not significantly differ between *Bt*- and non-*Bt*-foliage, neither did the number of offspring differ between the two treatments (Escher *et al.* 2000). The analysis of juveniles, however, revealed higher mortality rates of *P. scaber* reared on non-transgenic foliage compared to woodlice fed on transgenic plant material. These mortality rates, together with increased weight gain of adult woodlice fed on transgenic foliage, indicated higher nutritional quality of the transgenic maize used in the experiment (slightly lower C:N ratio, lower lignin content, higher content of soluble carbohydrates) (Escher *et al.* 2000). In a subsequent study, six non-*Bt*-maize varieties and two *Bt*-maize varieties were compared during a 20-day feeding experiment in the laboratory, with regard to consumption by *P. scaber* (Wandeler *et al.* 2002). The consumption of maize leaves differed between the eight maize varieties. Although *P. scaber* was found to feed significantly less on one of the two used *Bt*-varieties compared to its corresponding non-transgenic control variety, it was also found that the other *Bt*-variety was one of the preferred maize varieties when compared with all eight maize varieties evaluated. Overall, differences between maize varieties were found to have a stronger influence on consumption than a potential effect of the *Bt*-toxin alone. Differences in energy content were detected between the different maize varieties, but no correlation with the consumption rate was shown (Wandeler *et al.* 2002). In the most recent study, *P. scaber* was fed for 15 days on two different *Bt*-maize varieties expressing Cry1Ab (Pont & Nentwig 2005). ELISA analysis of the faeces revealed that depending on the maize variety, between 60 and 80% of the *Bt*-toxin was digested. Results of a bioassay further suggest that a part of the *Bt*-toxin taken up by primary decomposers is not digested and retains its insecticidal activity (Pont & Nentwig 2005).

7.3.4 Collembola and soil mites

No negative effects of the *Bt*-toxin Cry1Ab on collembolans and mites were found in two laboratory studies (Sims & Martin 1997, Yu *et al.* 1997). Microbially produced purified *Bt*-toxin was added at concentrations of 200 µg/g fresh weight to the diets of the collembolans *Folsomia candida* and *Xenylla goisea* for 21 days at temperatures of 19°C (Sims & Martin 1997). In the field, concentrations of *Bt*-toxins in plant material exposed to soil organisms are usually much lower, and are estimated to be less than 30 µg/g fresh weight (Sims & Martin 1997). The results showed no effects on adult survival or reproduction and are consistent with the findings of Yu *et al.* (1997) with *F. candida* and the soil mite *Oppia nitens* feeding on fresh cotton tissue expressing Cry1Ab. In the study by

Yu *et al.* (1997), organisms were fed on fresh and frozen six-week-old cotton leaves for around seven weeks at 21°C and no significant effects could be detected on oviposition, the number of eggs produced per female or final body length. Pre-market risk assessment studies submitted for regulatory approval of several *Bt*-maize and *Bt*-cotton varieties have not revealed any toxic effect of Cry1A proteins on *Folsomia candida* either (EPA 2001).

7.3.5 Earthworms

Impacts of *Bt*-maize expressing Cry1Ab on earthworms (*Lumbricus terrestris*) have been studied in the laboratory and under semi-field conditions by Saxena & Strotzky (2001a) and Zwahlen *et al.* (2003b). Both studies showed no consistent effects on *L. terrestris*. On the whole, laboratory experiments with adult earthworms feeding on *Bt*- and non-*Bt*-maize litter showed no significant difference in weight change between the two treatments. An 18% loss in weight in the *Bt*-treatment was observed only during a limited period towards the end of one study (Zwahlen *et al.* 2003b). Under semi-field conditions, no significant differences in growth patterns were observed in immature earthworms feeding on *Bt*- and non-*Bt*-litter (Zwahlen *et al.* 2003b). These findings are in agreement with earlier studies by Saxena & Stotzky (2001a), where, after 40 days exposure to root exudates or plant biomass of *Bt*- and non-*Bt*-maize, no significant differences were observed in the weight of *L. terrestris*. It was nonetheless evident that *Bt*-toxins were taken up as they were detectable in the casts as well as the guts of earthworms. Within two to three days after placing earthworms in fresh soil, the toxins, however, were cleared from the gut. Pre-market risk assessment studies submitted for regulatory approval have not revealed any toxic effect of Cry1A proteins on the earthworm *Eisenia fetida* either (EPA 2001).

7.4 Impacts on soil processes

Soil microbial communities carry out complex processes that are of major ecological and agricultural significance. One of their key functions in maintaining healthy soils is the regulation of nutrient cyclings including nitrogen mineralization and carbon cycling. To date, little research has been directed towards the influence of *Bt*-crops as well as the impacts of *Bt*-toxins on these soil processes (O'Callaghan *et al.* 2005). Concerns have been raised that *Bt*-maize may influence nutrient cycling by increasing the amount of residues returned to the soil, altering plant residue composition (Motavalli *et al.* 2004). Plant residue decomposition has therefore most often been chosen as an indicator of soil ecosystems functions. In addition to soil characteristics and the therein occurring microbial soil communities, plant residue decomposition is also influenced by chemical plant composition, which is known to differ among varieties (Mungai *et al.* 2005). Comparison of *Bt*- and non-*Bt*-maize residue composition have generated variable results. Depending on the variety, two *Bt*-maize varieties had higher starch and ligning content and lower protein and soluble N contents compared with non-transgenic maize (Masoero *et al.* 1999). Similarly, a 33–97% higher lignin content was found in ten *Bt*-maize varieties, either grown in a plant growth chamber or in the field, compared with their respective non-*Bt*-isolines (Saxena & Stotzky 2001b). In contrast, a lower C:N ratio and lignin content and a higher content of soluble carbohydrates were found in leaves of one *Bt*-maize variety compared with its corresponding non-transgenic maize variety (Escher *et al.* 2000), whereas no consistent differences in *Bt*- and non-*Bt*-maize residues composition were observed in a two year field study comparing five *Bt*-maize varieties with their non-transgenic isolines (Mungai *et al.* 2005). While Hopkins and Gregorich (2003) found no detectable difference between the decomposition rates of *Bt*- and non-*Bt*-maize, as

determined by CO_2 production, some studies observed lower decomposition of *Bt*-maize (Dinel *et al.* 2003, Stotzky 2004, Flores *et al.* 2005). These findings may be explained by a reduction in European Corn Borer damage or differences in the composition of *Bt*-maize residues that may increase the amount of undamaged, low N-containing residues remaining on or in the soil after harvest, thereby possibly reducing the rate of decomposition and nutrient mineralization (Motavalli *et al.* 2004). The results from a number of studies to date are contradictory, partly because observed differences in plant characteristics are not necessarily related to the inserted transgene (O'Callaghan *et al.* 2005) and the significance of the reported effects on soil processes is unclear. No conclusive research has yet been presented that currently commercially cultivated GM crops are causing significant effects on stimulating or suppressing soil nutrient transformations in field environments (Motavalli *et al.* 2004).

7.5 Conclusion on the ecological significance of impacts on soil ecosystems

Many of the studies referred to in this chapter have focused on the detection of differences between *Bt*- and non-*Bt*-crops and they have been able to detect some differences in the number of species and in the composition of microbial soil communities. The limited knowledge on the complex diversity of soil microorganisms does, however, not allow to determine the importance and the functional consequences of detected differences in soil microbial populations and it is thus not possible to put an ecological value on these differences. To date, no evaluation has yet been published on the ecological relevance of differences in populations, communities or processes in soil ecosystems due to the cultivation of GM crops. With the exception of Griffiths *et al.* (2005), observed differences have barely been compared with natural background variation, differences between conventional cultivars and crop systems, and impacts caused by routine pesticide application. In addition, knowledge gaps on the natural background variation occurring in agricultural systems still hinder the full interpretation of study results making a clear definition on what is considered an ecologically relevant effect on soil ecosystems difficult. Although a final conclusion can not be drawn, the scientific data obtained so far suggests that the effects owing to the cultivation of *Bt*-crops fall within the normal variation expected in agricultural systems and that they are not as large as those resulting from growing different (conventional) maize cultivars, crops, or as large as natural differences between sites or sampling occasions (Griffiths *et al.* 2005).

Summary: Impacts of GM crops on soil ecosystems

- *Bt*-toxins expressed in *Bt*-crops enter the soil system primarily via root exudation and via plant residues after harvest. Both degradation and inactivation of the *Bt*-toxin vary, depending on parameters such as temperature and soil type. Initial degradation of the toxin is rapid with a low percentage that may remain in the soil ecosystem following one growing season. It has been shown that *Bt*-toxins may bind to clay and humic acid compounds, however, several studies found no accumulation of *Bt*-toxins after several years of cultivation.
- Population sizes and community structure of soil organisms are subject to both natural variation and to variations caused by agricultural systems (soil type, plant age, crops, cultivars, crop rotation, and tillage practice). Neither laboratory nor field studies have shown lethal or sublethal effects of *Bt*-toxins on non-target soil organisms such as earthworms, collembola, mites, woodlice or nematodes.

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- Some differences between *Bt*- and non-*Bt*-crops in total numbers and community structure have been described for microorganisms. Many of these observations, however, are not statistically significant, transient, not related to the inserted transgene, or due to altered plant characteristics (e.g. lignin content).
- The ecological significance of the differences between *Bt*- and non-*Bt*-crops is not clear. Because most studies have not assessed the natural variation occurring in agricultural systems, it is generally difficult to establish whether the observed differences were exceeding this variation. The only study considering natural variation suggests that observed effects lie within this variation, and that the differences between conventional cultivars outweigh the observed influences of *Bt*-crops.

8 Gene flow from GM crops to wild relatives

The exchange of genes between crops and their wild relatives has always occurred ever since the first plants have been domesticated. Natural hybridization of crops and related plants is considered to have played an important role in both domestication of crops and the evolution of weeds (Conner *et al.* 2003). Surprisingly, gene flow from crops to wild relatives has only recently received major attention in the context of genetically engineered crops. Concerns have been raised that transgenes engineered into crops could be unintentionally introduced into the genomes of their free-living wild relatives (Ellstrand 2003). Two major concerns related to transgenes in natural populations will be addressed in this chapter:

(1) Could transgenes confer a benefit to **weedy relatives** (resulting in the evolution of so-called "superweeds") which could then become very difficult to control **in an agricultural environment**? Weedy relatives are species related to crops which may grow within the crop or may occur in peri-agricultural environments, such as field margins or road verges.
(2) Could **wild relatives growing in "natural" environments** suffer an increased risk of extinction due to hybridization with GM crops? Transgenic hybrids could become more competitive than the wild type. This would then lead to the extinction of the "wild type" occuring outside arable agriculture in semi-natural habitat-types such as grass- or woodland (e.g. clover, alfalfa and ryegrass).

It is generally agreed that the hazards related to gene flow from GM crops are linked to the introgression of transgenes into populations of wild relatives (Dale *et al.* 2002, Conner *et al.* 2003, Stewart *et al.* 2003, de Nijs *et al.* 2004, Hails & Morley 2005). There is little scientific support for the assertion that transgene dispersal is a hazard in itself. This matter will therefore not be specifically addressed in this study.

8.1 Principles of gene flow and fitness of transgenic hybrids

Transgene dispersal is often simply seen as pollen flow from the GM crop to its relative. The process of introgression, however, is not this simple and actually occurs in many steps involving several hybrid generations (Stewart *et al.* 2003). Gene flow can roughly be separated into two processes: hybridization and introgression. For hybridization to occur the transgenic crops and wild plants must grow within pollen dispersal distance, be sexually compatible, flower at the same time and viable pollen must be delivered to the stigma. Successful fertilisation of the embryo must then be followed by zygote and seed formation. Introgression requires the hybrid seed to germinate and the first filial generation (F_1) plant to establish and flower in order to further hybridise with members of the recipient population (Ellstrand *et al.* 1999, Stewart *et al.* 2003). F_1 hybrids must therefore persist for at least one generation and be sufficiently fertile to produce backcross hybrids. Finally, backcross generations must progress to the point at which the transgene is incorporated into the genome of the wild relative.

Apart from various biological factors, another important element determining the likelihood of transgene introgression is the occurrence of related species in the area where the crop is grown. Since most crops have been bred from wild plants it is not surprising that on a global scale nearly all crops may hybridise with a wild relative in some

part of their distribution range (Ellstrand *et al.* 1999). However, only a small fraction of the world's flora has been domesticated and in modern agricultural systems, many crops are grown outside the range of the wild relatives with which they might hybridise (GM Science Review Panel 2003). The potential for gene flow therefore varies from region to region. This chapter will mainly focus on oilseed rape (OSR) (*Brassica napus*) since this is currently the only crop where GM varieties are widely commercialized and where gene flow to wild relatives must be considered in Switzerland (Jacot & Ammann 1999). Another crop with a high potential for gene flow to wild relatives in Switzerland is alfalfa (Jacot & Ammann 1999). In September 2005, herbicide tolerant GM alfalfa has received commercial approval in the United States and in Canada (AGBIOS 2006), but there are no indications for commercial cultivation yet.

The key issue whether a weedy plant might evolve to a more competitive weed after hybridization with a related GM crop has occurred or whether a transgene might increase the competitiveness of wild relatives in natural ecosystems depends on two factors: (1) does the transgenic trait confer a selective advantage to the wild plant, and (2) is the trait able to subsequently establish in a natural population. Interestingly, with very rare exceptions, transgenic traits in plants are almost universally more or less dominant (because there are no corresponding alleles in wild plants, i.e. the "allele" in the wild plant is simply an empty location on the chromosome corresponding to the point of insertion in the transgenic plant – a situation called hemizygosity). In contrast, it appears that the majority of the traits that distinguish cultivated plants from their wild relatives are determined by recessive alleles (Ellstrand 2003). Due to their dominance, transgenes will be much more subject to natural selection, whereas the majority of crop alleles incorporated by traditional breeding will tend to be masked from natural selection when they introgress into natural populations. Fitness consequences of transgenes are therefore essentially depending on the character of the transgenic trait (see Box 5 for details).

> **Box 5: Transgenic traits and their potential fitness consequences under natural (field) conditions (adapted from Stewart *et al.* 2003)**
> - Transgenes that have a neutral effect on plant fitness might spread in natural populations, but would have no subsequent effect.
> - Genes with detrimental effects are selected against in neutral environments (including many traits associated with crop domestication, such as male sterility, altered fibre quality, changes in lignin biosynthesis).
> - Transgenes conferring HT or IR will vary in their fitness potential, depending on the invasiveness of the recipient species and on the level of natural control. Herbicide tolerance genes are selectively neutral in natural environments - HT is therefore unlikely to confer a benefit in natural habitats. Insect resistance genes, however, could increase fitness if the pest controls natural populations (Halfhill *et al.* 2002, Snow *et al.* 2003).
> - Transgenes changing environmental tolerance (e.g. cold, salt, drought tolerance) could extend the habitat range of the recipient wild species.

The presence of a transgene does not in itself appear to be generally beneficial or detrimental in hybrids (Ellstrand 2003, Hails & Morley 2005). The relative fitness of hybrids is depending both on the genotype and on the environmental conditions the hybrids are encountering. Transgenes that produce insect resistance (IR) will vary in their fitness potential – the common conclusion is that the transgenes will only confer a selective advantage if the fitness of wild populations is influenced by pests (Stewart *et al.* 2003,

Hails & Morley 2005). Some studies were able to confirm this hypotheses, e.g. F_1 hybrids of oilseed rape and *Brassica rapa* containing *Bt* genes were found to have a fecundity advantage under high insect herbivore pressure (Mason *et al.* 2003, Vacher *et al.* 2004). However, these experiments also suggested that, in the absence of herbivores, fitness costs occur, which consequently are negatively influencing the competitiveness of the transgenic hybrids (Hails & Morley 2005).

In most studies investigating the performance of transgenic hybrids between agricultural weeds and GM crops in semi-wild conditions, the hybrids were produced by artificial hybridization, i.e. they were crossed by hand pollination (Annex 1). Since many of these studies additionally manipulated the environmental conditions, it is difficult to judge how hybrids would behave under natural conditions (Hails & Morley 2005). Studies investigating fitness consequences under natural conditions show contradictory results. Transgenic hybrids of *Bt*-sunflower (where a *Bt*-transgene had been backcrossed into wild sunflower populations) exposed to natural levels of herbivory produced more seeds per plant due to reduced herbivory illustrating a selective advantage under realistic field conditions (Snow *et al.* 2003). In contrast, a series of gene flow studies have found that *weed x crop* hybrids are either less adaptive or equivalent to both the weedy and crop parent. Burke & Riesenberg (2003) found that the introduction of a disease-resistance transgene into wild sunflowers would not increase their fitness and that the transgene would diffuse neutrally after its escape. Similarily, hybrid populations of OSR x weedy *B. rapa* containing a *Bt cry1Ac* gene, performed worse or equivalent to non-transgenic *B. rapa* when competing against wheat in an experimental field trial (Halfhill *et al.* 2005). A possible explanation for the generally lower competitiveness of transgenic hybrids compared to the wild type could be the retention of crop-specific genes in the hybrids. Transgenic hybrid populations are genetically more crop-like than the wild-type populations. Many of the traits that are being added through genetic engineering, such as disease and insect-resistance, replace traits that have been lost in crops during domestication. However, these traits can still be found in related weeds, thus there seems to be little potential advantage associated with these kind of transgenes (Warwick & Stewart 2005).

8.2 Hybrids of oilseed rape becoming more competitive weeds in agricultural habitats

Commercial cultivation of GMHT oilseed rape (OSR) is to date the only situation, which could possibly lead to the introgression of herbicide tolerance genes into weedy relatives in Switzerland. Weedy relatives are species related to crops which may grow within the crop or may occur in peri-agricultural environments. Examples of weedy relatives of OSR include wild turnip (*Brassica rapa*), wild mustard (*Sinapis arvensis*) and charlock (*Raphanus raphanistrum*). Any transfer of herbicide tolerance to these cruciferous weeds could render their control more difficult in both oilseed rape and subsequent crops in a rotation. Farmers would then have to find an alternative herbicide or a new control method. The introgression of herbicide resistance genes into weedy relatives by gene flow should not be confused with the selection of herbicide resistant weeds by intensive herbicide applications. Although the immediate outcome would be the same, the cause is not (see chapter 10.1.2).

The probability of gene flow from OSR to *S. arvensis* (Moyes *et al.* 2002) and *R. raphanistrum* (Darmency *et al.* 1998, Gueritaine *et al.* 2002, Gueritaine *et al.* 2003) seems to be very low (Annex 1). The occurence of spontaneous hybrids in commercial fields is

therefore unlikely (Warwick *et al.* 2003, Daniels *et al.* 2005). Spontaneous hybrids between OSR and *B. rapa* are known to occur under field conditions with either species as the pollen donor (Annex 1; Hansen *et al.* 2001, Halfhill *et al.* 2002, Hansen *et al.* 2003, Warwick *et al.* 2003, Halfhill *et al.* 2004, Daniels *et al.* 2005). However, the transfer of herbicide tolerance genes from OSR to *B. rapa* seems to vary considerably in agricultural environments. To date, only two studies have discovered herbicide resistant F_1 hybrids between *B. rapa* and OSR under commercial agricultural cultivation conditions (Warwick *et al.* 2003, Daniels *et al.* 2005). In a Canadian study conducted in Québec, mean hybridization rates in feral populations of *B. rapa* were found to be 13.6% when sampled in or near a commercial field and 7% when sampled in two field experiments (Warwick *et al.* 2003). The higher frequency in commercial fields was explained to be most likely due to greater distances between individual *B. rapa* plants leading to higher pollen competition with OSR pollen. In contrast, in a similar study conducted during the Farm Scale Evaluations (FSE) in the UK, weedy *B. rapa* growing amongst OSR fields and within a 10 meter strip next to the crop edge had been sampled, and only two out of approximately 9500 seedlings were found to have incorporated the herbicide tolerance gene (Daniels *et al.* 2005).

The considerable differences in the hybridization rates found in the two studies have not been elucidated yet. They could possibly be due to several factors:

- Variations in the agricultural practice resulting in different amounts of *B. rapa* volunteers occurring as agricultural weeds

- Variations in the fertility of the OSR cultivars used (conventional varieties vs. varietal associations) resulting in different amounts of transgenic pollen

- Variations in the coincidence of flowering between both *B. napus* and *B. rapa*

Finally, if the hybridization rates found in the Canadian study are considered to be a critical issue, the results should also be discussed considering that GM herbicide tolerant *B. rapa* is also commercially grown in Canada for seed oil production for human consumption and seed oil and meal for livestock feed (AGBIOS 2006).

8.3 Transgenic hybrids outcompeting wild types in natural habitats

To date no long-term introgression of transgenes into wild populations leading to the extinction of any wild plant taxa has been observed (Ellstrand 2003, Stewart *et al.* 2003, Hails & Morley 2005). Hybridization-mediated environmental impacts from GM crops seem not to be any different from those of traditionally bred crops. Gene flow from traditional crops has on some occasions created problems by bringing wild relatives closer to extinction. However, there are only two known examples of crop-gene flow that have led to the evolution of decreased fitness in wild populations. Natural hybridization of an endemic wild rice species (*Oryza rufipogon* ssp. *formosana*) with cultivated rice (*Oryza sativa*) contributed to its extinction in Taiwan (Ellstrand 2003). Similarly, genetic pressure due to the cultivation of the purple flowering alfalfa (*Medicago sativa*) has lead to the disappearance of the yellow flowering wild type (*M. falcata*) from large areas in Switzerland (Rufener Al Mazyad & Ammann 1999).

Why the potential extinction of wild taxa by natural hybridization between GM crops and wild relatives has remained a rather theoretical risk as yet, could be due to the following reasons:

- **Fitness potential of current genetically engineered traits**

  Herbicide tolerant oilseed rape is currently the only commercial GM crop having a range of wild relatives in most of the areas where these crops are cultivated. However, HT genes are selectively neutral in an environment where no herbicide is applied. It is therefore unlikely that they confer a benefit in natural habitats (see Box 5). Transgenes that have the potential to increase fitness and competitiveness of transgenic hybrids in natural habitats include those conferring insect and disease resistance, drought and salt tolerance, and a suite of other fitness-enhancing traits that could be important in natural habitats (Stewart *et al.* 2003) (see chapter 8.1). Apart from insect resistance, no GM crops containing fitness-enhancing traits are commercially cultivated yet.

- **Cultivation range of insect-resistant GM crops**

  The only two insect resistant GM crops that have been widely commercially cultivated on a large scale are *Bt*-maize and *Bt*-cotton (see chapter 6.2.2). The movement of transgenes from these crops into weeds has been a significant concern during their pre-market risk assessment in the United States (EPA 2001). It was determined that wild species related to both crops, as found within the United States, cannot be pollinated by the crop due to differences in chromosome number, phenology (i.e. periodicity or timing of flowering) and habitat. The only exception would be a potential gene transfer from *Bt*-cotton to wild cotton relatives in Hawaii, Florida and the Caribbean. The U.S. EPA prohibited the sale or distribution of *Bt*-cotton in these areas. Neither *Bt*-maize nor *Bt*-cotton has therefore been cultivated in the range of origin of their respective wild relatives.

- **Overlap of agricultural and natural habitats**

  It can be expected that levels of hybridization are lower in natural than in agricultural habitats simply because the probability of crops and wild relatives occurring in close proximity (i.e. being separated by less than a few metres) is much higher in the latter case. In most agricultural landscapes there is a gradual transition from peri-agricultural to semi-natural habitats. A clear definition of what species are considered being "weedy" and "wild" relatives is therefore often lacking (see chapter 8.4). However, so-called wild populations are often growing in natural habitats which are more or less remote from agricultural fields. Because gene flow is a function of distance, i.e. hybridization is rapidly decreasing with increasing distance; the probability of transgenes introgressing into populations of natural habitats is certainly lower.

8.4 Conclusions on gene flow to wild relatives

There is general agreement that gene flow from GM crops to sexually compatible wild relatives can occur. Experimental studies have shown that GM crops are capable of spontaneously mating with wild relatives, however at rates on the order of what would be expected for non-transgenic crops (Ellstrand 2003). Much empirical information about crop-wild relative hybridization is now available (de Nijs et al. 2004) indicating that such hybridization occurs when sexually compatible wild relatives are present in close proximity to the crop, albeit at low (and variable) rates (Stewart et al. 2003). Hybridization between conventional (non-GM) crops and their wild relatives has occasionally caused problems in ecological and evolutionary time (see chapter 8.3). There is no evidence as yet that GM crops pose any greater risk than do non-GM crops, but our knowledge of the fitness consequences of transgenes in wild populations is incomplete (Hails & Morley 2005). It is difficult to judge a priori whether a transgenic phenotype will have a special fitness advantage relative to a non-transgenic counterpart – and if an advantage exists,

whether this will result in increased weediness. The data published so far indicate that serious ecological consequences have not been observed (Stewart et al. 2003).

We believe that it is important to consider the meanings of the terms "wild plant" and "weed": Wild plants are essentially free-living, i.e. they are plants that grow and reproduce without being deliberately planted and cared for (Ellstrand 2003). On the other hand, weeds are popularly defined as plants growing in the wrong place, causing damage and suppressing cultivated plant species (Ammann *et al.* 1996). We believe that when discussing gene flow from GM crops, a distinction has to be made between potential effects occurring within agricultural environments and effects, which could occur in natural habitats. The first case (still being the most conceivable) can rarely be described as being of ecological relevance since weeds are plants competing with the crop or harming the harvested product. Farmers tend to get rid off weeds and increased weediness is therefore primarily an agronomic or economic concern. Furthermore, increased weediness is in most cases not a result of genetic engineering but more of crop and herbicide management. In addition to the low probability that increased weediness due to gene flow will occur on a large scale and for a multitude of crops, farmers can generally choose among a set of options to control or manage weeds. The probability that ecologically relevant effects will occur in natural habitats is even lower and remains, as yet, purely theoretical.

Summary: Gene flow from GM crops to wild relatives

- The empirical information about crop-wild relative hybridization now available indicates that hybridization occurs when sexually compatible wild relatives are present in close proximity to the crop, albeit at low (and variable) rates.
- In most studies investigating the performance of transgenic hybrids between agricultural weeds and GM crops in semi-wild conditions, the hybrids were produced by artificial hybridization, i.e. they were crossed by hand pollination. Since many of these studies additionally manipulated the environmental conditions, it is difficult to judge how hybrids would behave under natural conditions.
- Spontaneous hybrids between oilseed rape *(Brassica napus)* and wild turnip *(Brassica rapa)* are known to occur under field conditions with either species as the pollen donor. Two studies have discovered herbicide resistant F_1 hybrids of *B. rapa* and oilseed rape under commercial agricultural cultivation conditions. The transfer of herbicide tolerance genes from oilseed rape to *B. rapa* seems to vary considerably in agricultural environments. The reasons for the different hybridization rates found in the field are unclear.
- In natural habitats, no long-term introgression of transgenes into wild populations leading to the extinction of any wild taxa has been observed to date

9 Invasiveness of GM crops into natural habitats

The awareness of the problems that have sometimes attended the deliberate or accidental introduction of non-native species into new environments has a long history (Elton 1958). Invasions have been recognized in a growing number of environments as being serious threats to the preservation of what we choose (by our choice of time scale) to be regarded as native fauna and flora (Sakai *et al.* 2001, D'Antonio & Meyerson 2002, Levine *et al.* 2003). Although the great majority of accidental introductions undoubtedly failed to become established, a substantial number became established, and some of these became serious pests (Levin 1988). Not surprisingly, the concern of GM crops invading natural habitats was brought up early in the discussion on potential environmental risk related to the release of GM crops (Levin 1988).

9.1 The "exotic species model" vs. the "crop model"

Basically, two principal models have been influential in considering the potential risk of invasiveness of GM crops: (1) the exotic species model and (2) the crop model. The **exotic species model** hypothesizes that about ten percent of all introduced non-native species will establish, and about ten percent of those will become weeds (Williamson 1993). In addition, ten percent of these weeds become introduced in the sense of resulting in feral individuals (Williamson 1994). According to this model, roughly 0.1% of all released GM crops would therefore result in feral individuals becoming serious pests (Williamson 1993, 1994). The exotic species model may offer a methodological approach to make potential ecological long-term prognosis based on extensive historic ecological data when releasing GM crops (Sukopp & Sukopp 1993). Risk assessment procedures are, however, not capable of observing naturalization processes of species for decades and centuries. To make statements on the short-term ecological risk of a single, precisely characterized GM crop, it seems more reasonable to compare the GM crop to the ecological behaviour of the conventionally bred crop, which has been cultivated for a long time (Sukopp & Sukopp 1993). The more recent **crop model** assumes that GM crops will behave in much the same way as conventional crop plants except for the GM trait that may influence fitness (Crawley *et al.* 2001). If GM crops exhibit traits that are not expected to increase their fitness in semi-natural habitats, they probably behave like the non-GM crop with respect to invasiveness. In purely ecological terms, it is impossible to detect a particular risk in the release of transgenic plants that could be assumed to be absent in the case of conventionally bred plants (Sukopp & Sukopp 1993). The authors further state that "if we accept the necessity of a risk assessment in the case of the deliberate release of transgenic plants, we should also assess the potentially harmful ecological effects of growing conventionally bred plants" (Sukopp & Sukopp 1993).

9.2 Domesticated crops, agricultural weeds and ferality

Both domesticated crops and agricultural weeds have arisen from wild plants, however domesticated crops differ from weeds, as they are mostly human inventions selected for certain traits through thousands of years. This selection process has led to a number of domestication traits (e.g. self-fertility, non-shattering seeds or seedpods) which are typically shared by a wide variety of crops. Weeds, in contrast, are not simply wild plants that interfere with the growth of domesticated crops. Weeds have indirectly or inadvertently been selected by farming practices, selecting for plants with specific traits such as

staggered germination, rapid early season growth, continuous flowering and seed production. Most common distinctive attributes of weeds have been bred out of crop plants over thousands of generations, and these characters are not candidates for gene transfer back into crops, because they would severely reduce their agronomic performance for modern farming practice (Conner *et al.* 2003). If modern crop cultivars no longer possess weedy characteristics, their ability to become weeds is severely hindered in the absence of gene introgression of these weedy traits from wild races (Conner *et al.* 2003).

Wild plants have a genetic architecture that enables them to grow independently of humans for propagation and independently of human-disturbed habitats. In contrast, both crops and weeds have a dependency on human-disturbed habitats to grow. Weeds generally exhibit a preference for disturbed habitats such as cultivated fields, field margins, gardens and roadsides. Most weeds are dispersing without human intervention, whereas crops are highly dependent on humans for propagation. Furthermore, unlike wild plants occupying a variety of ecological niches, agricultural weeds are narrowly selected for inhabiting cropping systems under specific management practices. Crops and agricultural weeds occupy the same habitat and weeds are often associated with a specific crop. At first glance, it would appear that crops becoming feral should be widespread, because many crops have the capacity to volunteer in subsequent crops. In spite of the apparent advantages conferred to volunteers, the modes of weed evolution – crop-turns-into weed on its own (endoferality) and crop-mediated ferality through gene flow from related weeds (exoferality) – are rare in nature (Warwick & Stewart 2005). This may well be due to crop rotation, where volunteers or hybrids are no longer competing with the parent crop, but must compete with a different crop and agronomic procedure. But perhaps transgenes will change all that. There are theories, being the antithesis of the crop-like domestication model, arguing that the hemizygosity of transgenic traits (see chapter 8.1) could lead to a specialized weediness syndrome (Ellstrand 2003). Because most crops and weeds can interbreed somewhere in the world, this would lead to more problematic weeds. In order to determine if transgenes could ameliorate ferality in a crop or in crop-weed hybrids, it is therefore important to examine the degree of crop domestication, the most important weeds associated with important crops, and the role transgenes might play in dedomestication.

9.2.1 Degree of crop domestication

Crops vary in their degree of domestication, i.e. in the proportion of domestication traits compared to wild traits. The continuing adaptation of plants to human cultivation is known as the domestication syndrome; some crops may only have a few domestication traits, whereas other crops may have all or most of the features (Warwick & Stewart 2005). Virtually all important crops have a high degree of domestication because they were selected for traits that lead to a favourable food and fiber production. Crops such as maize and wheat never existed in nature, and rice and oilseed rape have undergone significant breeding compared to their so-called natural form. Generally, domesticated crops have been deprived of some of their natural resistance to environmental conditions and tend not to be competitive in the wild. However, certain categories of transgenic crops could pose special risks, particularly those that are hardy, perennial, competitive, open-pollinating, prolific, have a wide range of relatives with which they hybridize, and have an ability to colonize a range of natural and semi-natural habitats (Warwick & Stewart 2005). Examples of such plants include grasses and pasture species. Although genetically modified alfalfa and GM creeping bentgrass (*Agrostis stolonifera*) have obtained regulatory approval in the United States, no genetically modified varieties have been commercialized yet (AGBIOS 2006).

Weedy characteristics of maize

Maize putatively arose from a single wild teosinte strain in southern Mexico. Unlike its progenitor, maize carries its seeds on an ear that eases harvest, a trait that also prevents it from escaping cultivation and becoming feral. Maize is therefore highly domesticated and does not form feral populations (and only rarely volunteers) (Warwick & Stewart 2005). Although gene flow is primarily unidirectional from teosinte to maize, maize does not have any weedy derivatives.

Weedy characteristics of soybeans

Soybeans are not generally considered a serious volunteer weed problem as exemplified by the lack of published literature (Owen 2005). Seeds lost during harvest do not overwinter particularily well in the main growing areas of the U.S., and if volunteer plants develop in the rotational crop, the losses due to interference are minimal. Ferality in soybeans is not a serious agricultural issue. Although there are genetically compatible species for the potential introgression of traits, the species do either not occur in important soybeans production areas (e.g. wild soybean and soybean production in the Western Hemisphere), or they do not have a reported competitive ability to represent an agronomic threat (e.g. wild soybean in China) (Owen 2005).

Weedy characteristics of oilseed rape (OSR)

Due to its relatively recent domestication, compared to the highly domesticated cereal crops, OSR still retains more weedy characteristics than other crops. Oilseed rape is a crop that regularly forms volunteer populations due to extensive seed shattering and secondary seed dormancy (see chapter 8.2). The size and the shape of its small seeds contribute to a relatively easy self-burial in the soil, which facilitates survival in the seed bank (Hall *et al.* 2005).

9.2.2 Ferality and survival of OSR in semi-natural habitats

The life span of feral (non-transgenic) OSR populations in disturbed habitats (e.g. along roadsides) seems to vary between one to four years in the UK (Crawley & Brown 1995, 2004), and eight to nine years in France (Pessel *et al.* 2001). It was suggested that the feral OSR populations were either deriving from spillages from farm machinery and trucks or be the result of late germinations of dormant seeds (Pessel *et al.* 2001). It is interesting to note that both surveys were carried out in areas where OSR was present with a high proportion due to spillage of seeds during transportation to either a silo or a processing plant. Because road verges are often mowed every year, it seems that a large number of OSR seeds are regularly spread from trucks after harvest (Pessel *et al.* 2001). The invasive potential of OSR outside of agricultural fields is more likely to be influenced by active dispersal of seeds by man than by pure biological evasion, because the invasive potential of OSR seems to be limited. Like many annual weeds, OSR depends critically on disturbance; in undisturbed habitats it is generally out competed by perennials (Claessen *et al.* 2005a). The most frequently observed pattern in the OSR populations in the UK study involved a classic casual dynamic, i.e. the casual populations did not exhibit self-replacing dynamics. The populations lasted for just one to two years before they were locally extinct. This was most likely caused by interspecific competition from perennial grasses eliminating suitable sites for recruitment from OSR seed. Recruitment was only possible in habitats following soil disturbance and seed spillage from trucks in transit to the processing plant (Crawley & Brown 2004). It was also found that in the case of roadside OSR, dispersal is unlikely to result in permanent, global persistence at the metapopulation level, because this would require unrealistically high dispersal rates (Claessen *et al.* 2005b).

9.3 The influence of transgenic traits on persistence and ferality

9.3.1 Multiple herbicide resistances in OSR volunteers

Gene flow between different transgenic OSR growing in habitats which are frequently disturbed (such as road verges) has commonly been part of the discussion on environmental effects of GM crops, especially in Canada. There are three types of GM herbicide tolerant OSR commonly grown in Canada: glyphosate (counting for 59% of the total acreage in 2001) as well as glufosinate-resistant varieties (16%) – both obtained by genetic engineering – and a non-transgenic imidazolinone-resistant type (25%) (Beckie *et al.* 2004). It was conceived that the transfer of herbicide tolerance genes between varieties of OSR through gene flow may result in volunteers resistant to two or more herbicides, which could pose agronomic problems in volunteer plant control. After three years of commercial cultivation of GMHT OSR, two triple-herbicide resistant volunteers were reported at a field site in Western Canada (Hall *et al.* 2000) and a study at 11 sites in Saskatchewan, Canada, reported double-resistant OSR volunteers (Beckie *et al.* 2003). The results of both studies suggest that HT gene stacking can occur in OSR volunteers. This is not surprising, given the outcrossing potential of OSR, the large acreage of GMHT OSR in Western Canada, and the potential seed bank life leading to the incidence of OSR volunteers (Hall *et al.* 2000, Beckie *et al.* 2004, Legere 2005). Rotations including many GMHT crops having the same trait (e.g. glyphosate tolerance) may result in various crop volunteers resistant to the same herbicide and thus make certain cropping systems fragile (Legere 2005). However, there is no evidence at present that the extensive cultivation of GMHT OSR over seven years in Western Canada has resulted in an increase of volunteer OSR that would have been caused by the herbicide-tolerance traits (Hall *et al.* 2005). Extensive weed population monitoring has been conducted in thousands of fields and will continue to play an important role in assessing populations of herbicide-tolerant volunteers, weed population shifts, and changes to weed biodiversity due to GMHT crops. The lack of reported multiple-resistant volunteers suggests that these volunteers are being controlled by chemical and non-chemical management strategies, and are therefore not an agronomic concern to most producers (Hall *et al.* 2000, Hall *et al.* 2005). The multiplicity of herbicides available ensures that HT gene-stacked volunteers are not an agricultural problem. In Canada, there are over 30 registered herbicides to control single- or multiple-resistant GMHT OSR in cereals, the most frequent crop to follow OSR in a typical 4-year rotation (Beckie *et al.* 2004). Although not all volunteer OSR is killed by the herbicide application, most survivors are affected by the combination of crop competition and partial herbicide control that reduces seed set. In all crops except field peas, herbicides control herbicide-tolerant OSR because glyphosate and glufosinate are not used in crops other than OSR at this time in Western Canada (Hall *et al.* 2005). Furthermore, there are a multitude of cultural and mechanical practices that are recommended to growers to manage multiple-GMHT OSR volunteers. These include (Beckie *et al.* 2004) (1) leaving seeds on or near the soil surface as long as possible after harvest because a high percentage will germinate in the fall and be killed by the frost; (2) using tillage immediately before sowing; (3) silaging and green manuring to prevent seed set in volunteers; (4) isolating OSR fields with different HT traits; (5) following OSR with a cereal crop and rotating OSR in a 4-year crop rotation; (6) scouting fields for volunteers not controlled by weed management; (7) using certified seed and (8) reducing seed loss during harvest.

9.3.2 Invasiveness of transgenic crop varieties into semi-natural habitats

Not many experimental studies have been performed comparing the invasiveness of transgenic crop varieties to non-transgenic varieties. In an early study, population dynamics of GMHT OSR with a resistance to glufosinate and conventional OSR were estimated

over a 3-year period in twelve natural habitats and under a range of climatic conditions (Crawley *et al.* 1993). There was no evidence that genetic engineering for herbicide tolerance increased the invasive potential of OSR in undisturbed natural habitats. Furthermore, there was no evidence that transgenic OSR was more invasive or more persistent in disturbed habitats compared to their conventional counterparts. In general, the transgenic lines performed even less well than the non-transgenic lines. A more recent study compared four different crops (both conventional and GM) grown in twelve different habitats and monitored their performance over a period of ten years (Crawley *et al.* 2001). In no case the GM crops (OSR and maize expressing tolerance to glufosinate, sugar beet tolerant to glyphosate, and two types of GM potato expressing either the *Bt*-toxin or a pea lectin) were found to be more invasive or more persistent than their conventional counterparts.

9.4 Conclusions on the invasiveness of GM crops into natural habitats

In contrast to the theory claiming that the hemizygosity of transgenic traits could lead to an increased weeediness syndrome in GM crops (Ellstrand 2003) (see chapter 9.2), it seems that most crops generally stay domesticated (Warwick & Stewart 2005). Certain crops, such as maize, have a fixed suite of domestication genes and they are maladapted to survive without human intervention. Other crops, such as OSR, are sexually compatible to important weed groups. Although such related weeds could contribute weediness traits to crop-weed-derived feral forms, it seems that weeds generally do not arise by crop x weed hybridization, but by other means.

Despite the extensive commercial cultivation of GMHT OSR in western Canada for several years, there is currently no risk of GMHT OSR becoming feral. This is due to its lack of persistence in the seed bank, the redundant and repetitive control of volunteer weeds in subsequent crops, the absence of persistent populations in ruderal areas, and the limited occurrence of weedy relatives with a potential for hybridization (Hall *et al.* 2005).

Dedomestication of crops and associated ferality appears to be restricted to only a few crop groups. They are only of minor importance globally with regard to invasive weed problems especially compared to other plant groups (Warwick & Stewart 2005). Globally, the feral plants that cause much of the economic damage are imported horticultural plants (Sakai *et al.* 2001, D'Antonio & Meyerson 2002, Levine *et al.* 2003). Unlike annual crops, these horticultural plants are mostly perennials that have extensive sexual and asexual reproduction.

Summary: Invasiveness of GM crops into natural habitats

- There is no evidence at present that the extensive cultivation of GMHT OSR over seven years in Western Canada has resulted in a widespread dispersal of volunteer OSR carrying herbicide-tolerance traits.
- Although one study found triple-herbicide resistant OSR volunteers in Western Canada, and another study reported double-herbicide resistant volunteers, the general lack of reported multiple-resistant volunteers suggests that these volunteers are being controlled by chemical and non-chemical management strategies, and are therefore not an agronomic concern to most producers.
- Despite the extensive commercial cultivation of GMHT OSR in Western Canada for several years, there are currently no indications of GMHT OSR becoming feral.

10 Impacts of GM crops on pest and weed management

Environmental impacts due to crop management changes are usually difficult to assess because they are often caused by many interacting factors and do only show up after an extended period of time. Not surprisingly, the impacts of modern (non-GM) agriculture on biodiversity were only revealed years after these techniques had been introduced (see chapter 5.1). Considering the widespread effects modern agricultural systems had in the last decades, changes in management practices are probably among the most influential factors that could lead to biodiversity changes. It appears that concerns related to crop management changes are perceived more strongly and are judged to be more important since the adoption of GM crops and that these concerns were less prevalent in the past. It is, however, crucial to bear in mind that management changes are not exclusively limited to the adoption of the GM technology. They can occur in any (non-GM) crop management strategy, and could, for example, be caused by the adoption of new pesticides, cultivation techniques or crop varieties.

10.1 Weed management changes related to GM herbicide-tolerant crops

10.1.1 Shifts of weed populations and potential impacts on biodiversity

The impacts on farmland biodiversity due to the use of genetically modified herbicide-tolerant (GMHT) crops are currently discussed in two contrasting matters. While there are concerns that the control of weeds in GMHT crops using broad-spectrum herbicides might be so efficient that long-term declines in weeds could lead to the decline of wildlife depending on them (Watkinson *et al.* 2000, Heard *et al.* 2005), others suggest that GMHT crops might ameliorate farmland biodiversity by delaying and reducing herbicide use, and even allowing weeds and associated wildlife to remain in fields longer (Firbank & Forcella 2000, Dewar *et al.* 2003, May *et al.* 2005).

The concern that declines in weed number could have adverse effects on farmland biodiversity received major public attention due to the interpretations of the results of the Farm Scale Evaluations (FSE) performed in the United Kingdom (see Box 6 for details). The FSE were able to show that the biomass of weeds was reduced under GMHT management in sugar beet and oilseed rape and increased in maize compared with conventional treatments. However, the invertebrate groups assessed (herbivores, detritivores, pollinators, predators and parasitoids) were much more influenced by season and by crop type than by the GMHT management (Hawes *et al.* 2003). The abundance of many invertebrate groups increased two-fold to five-fold between early and late summer, and differed up to 10-fold between crops, whereas GMHT management superimposed relatively small (less than twofold), but consistent, shifts in plant and insect abundance.

The results of the FSE led some groups to the rather simplistic conclusion that the use of GMHT crops generally leads to lower weed and insect densities, consequently affecting farmland biodiversity and especially bird populations. Although the FSE were one of the most extensive ecological studies ever conducted, they were not without limitations (Chassy *et al.* 2003, Freckleton *et al.* 2003). As the authors of the FSE stated, "the FSE addressed one particular environmental risk of one particular trait in one particular agro-ecosystem, and the results should not be extrapolated to other socio-environmental

systems" (Firbank *et al.* 2003). There are two important limits that should be critically discussed:

- **Extrapolation of the results from the farm to the landscape level**

The effects observed in the FSE were restricted to the field-scale. The importance of weeds in oilseed rape crops as forage resources for bees and butterflies, for example, is as yet uncertain (Bohan *et al.* 2005). Their importance largely depends on the availability of alternative food resources in adjacent habitats, and the situation would only be critical during summer if forage reductions would occur over large contiguous areas. Taking into account that all three crops occupied less than 15% of the total arable field surface of Great Britain in any year (Squire *et al.* 2003), it is unclear if these effects would occur at the landscape-level and how significant they would be. A major factor in the decline in farmland biodiversity over the last decades has been the loss of more specialized taxa (Robinson & Sutherland 2002). Thus, many of the birds and butterflies that declined markedly in the period prior to 1970 were dependant on areas of extensive low-input cultivation or the presence of non-cropped habitat. In general the plants currently common on arable land are found in a wide range of other habitats. Similarly, butterflies as well as the non-declining farmland birds now typical of farmland in Britain are those that tend to be habitat generalist (Robinson & Sutherland 2002). More intensive field management, degradation in habitat quality, and increasing habitat homogeneity (across all-scales) are currently the most important drivers of biodiversity loss.

- **Consequences of the cropping and weed management system applied**

The FSE assumed that no other changes in field management will occur other than the GMHT crops replacing present non-GM varieties in a proportion of fields (Squire *et al.* 2003). The results are therefore linked to the weed management

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Box 6: The Farm Scale Evaluations (FSE) of the environmental impact of growing GMHT crops

The FSE were designed to test whether there is a difference in the biodiversity of weeds and invertebrates between GMHT crops and conventionally managed crops. The crops assessed included sugar beet, maize, as well as winter and spring oilseed rape. The studies were carried out over three years in more than 60 fields per crop. Fields were divided into two, one-half was sown with a conventional crop, and the other with a GMHT crop (1,2).

Main results:

- Differences in biodiversity between crops exceeded differences between GMHT crops and conventional crops (3-8). Weeds and some invertebrate groups were more abundant in oilseed rape (both GMHT and conventional) than in sugar beet or maize.

- In all three GMHT crops, the weed number and biomass was higher in early season (3,8,9). Following application of the broad-spectrum herbicides to the GMHT crops, the effect was reversed in both GMHT sugar beet and oilseed rape resulting in lower late-season biomass and seed rains of weeds. Weed control in GMHT maize was lower and weed numbers were therefore higher in GMHT maize throughout the season (3,4,8,9).

- As a result of lower abundance of flowering weeds, lower numbers of butterflies and bees were found in both GMHT sugar beet and oilseed rape; whereas generally higher number of invertebrates were found in GMHT maize (5,6,9).

- Higher detritivore (collembola) densities were found in GMHT sugar beet and oilseed rape, as well as in conventional maize, as a result of higher weed detritus (5,6,9).

- The effects on invertebrates and vegetation of field margins differed between the three crops, with less flowering and butterflies on margins of GMHT oilseed rape and sugar beet, but more flowering on GMHT maize margins, yet no butterfly differences for this crop (7).

- GMHT crops generally received only one herbicide active ingredient per crop, later and fewer herbicide sprays and a less active ingredient (for beet and maize) than the conventional treatment. In oilseed rape, a low input crop, the amount of active ingredient used did not differ significantly between GMHT and conventional crops (2).

1) Squire *et al.* (2003)
2) Champion *et al.* (2003)
3) Heard *et al.* (2003b)
4) Heard *et al.* (2003a)
5) Brooks *et al.* (2003)
6) Haughton *et al.* (2003)
7) Roy *et al.* (2003)
8) Hawes *et al.* (2003)
9) Bohan *et al.* (2005)

system practised in the FSE, for both conventional and GMHT systems. Highly effective weed control practices, such as those chosen for the GMHT crops in the FSE, lead to low numbers of weed seeds and insects. In turn, fewer insects and decreased weed seed might reduce the numbers of birds that depend on these insects and seeds as a food source (Chassy *et al.* 2003). However, other weed management systems than the one used in the FSE are possible. The use of GMHT technology in the U.S. and in Canada was accompanied by a series of management changes including the adoption of conservation tillage practices, which are considered to have several environmental benefits (Carpenter *et al.* 2002, Phipps & Park 2002) (see chapter 11.2). These include beneficial impacts on farmland biodiversity, because conservation tillage results in a greater availability of crop residues and weed seeds improving food supplies for insects, birds, and small mammals (Holland 2004). Similarly, studies conducted in the UK have shown that alternative scenarios to those resulting from the FSE are possible for GMHT sugar beet (Dewar *et al.* 2003, May *et al.* 2005). GMHT sugarbeet allows to choose an optimal application time and to reduce the number of herbicide sprays, resulting in environmental benefits compared with the conventional practice. Depending on the herbicide management chosen, it can either enhance weed seed banks and autum bird food availability, or provide early season benefits to invertebrates and nesting birds (May *et al.* 2005).

In conclusion, the FSE have not produced evidence for any new environmental damage as a result of GM technology. The reductions in invertebrate abundance result solely from the increased control of weeds, and the FSE appear to show that introducing GMHT crops is equivalent to the introduction of a new, very effective herbicide (Freckleton *et al.* 2003). Weed populations are the result of the herbicide management strategy, not the GM status of a crop (Chassy *et al.* 2003).

10.1.2 Selection of resistant weeds by intensive herbicide applications

The wide adoption of GMHT crops raised concerns that the increasing applications of one herbicide will rapidly enhance the evolution of herbicide-tolerant weed populations. However, independently from the adoption of GM crops, a number of changes have occurred in conventional agricultural systems during the past decades, which resulted in significant impacts on weed communities. The most important selective forces on a weed community in a crop rotation system are tillage and herbicide regime. Most of the resistant weed biotypes evolved without the selection pressure resulting from the adoption of GM herbicide-tolerant crops. Numerous weed species have evolved resistance to a number of herbicides in many, if not most, agricultural systems long before the introduction of GMHT crops (Owen & Zelaya 2005, Heap 2006). The commercialisation of herbicides inhibiting acetolactat synthase (ALS), for example, induced the evolution of herbicide-resistant biotypes in over 90 weed species, while 65 weed species have evolved resistance to atrazine (Owen & Zelaya 2005, Heap 2006). It seems that tolerance to glyphosate, in contrast, is less likely to develop in weed species (and in volunteers) than tolerance to other herbicides, as a result of its chemical properties and its mode of action (Bradshaw *et al.* 1997, CFIA 2003). After almost three decades of glyphosate use tolerance to glyphosate has only been reported in eight weed species worldwide (Heap 2006).

The experiences available from regions growing GMHT crops on a large-scale confirm that the development of herbicide-resistances in weeds is not a question of genetic modification, but of the crop- and herbicide management applied by farmers. Despite the extensive cultivation of GMHT oilseed rape in Canada, no weed species has so far been observed being tolerant to the herbicides glyphosate and glufosinate (CFIA, 2003).

Although no long-term studies have been conducted, no significant shifts in weed popu-lations and no major difficulties in the management of weeds in agricultural settings have been attributed to the widespread cultivation of GMHT oilseed rape in Canada either. This is, in part, certainly due to farmers rotating both their crops and the herbicides they use for weed and volunteer control. In the United States, in contrast, glyphosate has been used before the introduction of GMHT varieties in combination, or in sequence with other herbicides in continuously cultivated no-tillage soybean fields. With the wide-spread use of GMHT soybeans, many fields have been treated only with glyphosate, which increased the pressure for the selection of resistant weed biotypes. As a conse-quence, within three years after the introduction of GMHT soybean varieties, glyphosate-resistant horseweed (*Conyza canadensis*) has been detected (VanGessel, 2001). It is clear, that the continuous application of the same herbicide in one particular crop over multiple years without applying appropriate crop rotation will inevitably lead to the selection of herbicide-tolerant weeds. The limited number of herbicides used results in greater selec-tion pressure on the weed community. Unfortunately, the manner of use of glyphosate in the Midwestern United States makes the evolution of herbicide resistances inevitable. Glyphosate-resistant weeds have been described by some as "super weeds", and there have even been inferences that glyphosate-resistant weed presence could reduce farm-land value. Although farmers have to add another herbicide to glyphosate to control the resistant weed species, there are alternatives to glyphosate for most weed species that are highly effective and provide good flexibility in application timing. There is, however, no question that glyphosate-resistant weeds will increase the costs of weed manage-ment for farmers. A more costly scenario would involve a weed for which the alternative herbicides have limited flexibility in application timing. In this situation, the loss of appli-cation flexibility would present a greater cost to many farmers than the additional herbi-cide expense.

In conclusion, the simplest way for farmers to reduce selection pressure placed on weeds by glyphosate is to avoid planting continuous glyphosate-resistant crops and to annually rotate the herbicides used. Such procedures are in fact part of any reasonable herbicide resistance management strategy that should be followed by farmers and that are recommended by regulatory agencies in Europe and in North America, as well as by the industry (EPA 2006, Health Canada 2006, HRAC 2006).

10.1.3 Changes in herbicide use due to GMHT crops

There are many criticisms arguing that the adoption of GMHT crops would generally lead to an increased use of herbicides. Studies can be found to support this view (Ben-brook 2001, 2003), but there appear to be more studies that support a small but statisti-cally significant reduction in herbicide use (Carpenter *et al.* 2002, Fernandez-Cornejo & Mc Bride 2002, Brimner *et al.* 2005, Brookes & Barfoot 2005). Because the reduction var-ies between crops and regions, it is however difficult to draw a general conclusion. The adoption of GMHT varieties of oilseed rape in Canada, for example, has been associated with a reduction in the amount of herbicide used per hectare as well as a decline in the potential environmental impact of chemical weed management (Brimner *et al.* 2005). The average soybean herbicide application rates, in contrast, have slightly increased by 3% since the introduction of GMHT soybean in the U.S. (in terms of active ingredients per acreage) (Carpenter *et al.* 2002, Fernandez-Cornejo & Mc Bride 2002). However, it would be insufficient to assess herbicide use only by comparing the quantities of herbi-cides applied, even if expressed in the total amount of active ingredient. Beside net changes in the amounts used, the adoption of GMHT crops has more precisely resulted in a change in the mix of herbicides used. The assessment of this change, however, is not

as straightforward as it may seem, since toxicity and persistence in the environment vary across pesticides. Assessing herbicide changes relying purely on the amounts used, would assume that the same amount of any two ingredients has equal impact on human health and the environment, while in fact the various active ingredients in use in herbicides vary widely in toxicity and in persistence in the environment. The adoption of GMHT crops has allowed farmers to use herbicides (glyphosate and glufosinate) that are at least three times less toxic to humans and to the environment than the previously used (Fernandez-Cornejo & Mc Bride 2002, Duke 2005).

In some countries, especially in South America, the adoption of GMHT soybeans increased the volume of herbicides used relative to the amounts used before GMHT adoption (Trigo & Cap 2003, Brookes & Barfoot 2005, Qaim & Traxler 2005). This is largely due to the fact that the GMHT technology has accelerated the switch from a conventional tillage system (where no or less herbicides were used because weeds were mainly ploughed into the soil) to a conservation tillage system. The increase in the net volume of herbicides used should, however, be placed in the context of the environmental benefits of the new conservation tillage systems (see chapter 11.2).

10.2 Pest management changes related to GM insect-resistant crops

10.2.1 Insecticide use changes due to *Bt*-maize

Although the European Corn Borer (ECB) is a serious insect pest of maize causing considerable losses per year, only 5–8% of the maize area in the U.S. was sprayed with insecticides against ECB before the adoption of *Bt*-maize. This is mainly due to problems in assessing the correct time of spraying, because the larvae spend part of their development inside maize stalks where pesticides may not be effective (EPA 2001, Phipps & Park 2002). Insecticide use on maize is therefore largely to control soil pests, rather than the *Bt*-maize target pests. Large changes in insecticide use due to the adoption of *Bt*-maize were therefore not to be expected. Nevertheless, there exist disagreements over the interpretation of the impact of *Bt*-maize adoption on pesticide use data. An analysis performed by U.S. EPA indicated a reduction of about 1.6 million ha treatments with insecticides for all pests in maize (EPA 2001), a finding confirmed by a study concluding that insecticide use to control the ECB dropped by 1.5% between 1995 and 1999 (Carpenter & Gianessi 2001). One analyst concluded that insecticide applications targeting directly the EBC raised from 4% of the area treated to 5% in 2000 (Benbrook 2001). Considering the small increase reported in the last study, as well as the single year-date, a conclusion that *Bt*-maize generally leads to higher insecticide use is, however, little convincing. The adoption of *Bt*-cotton has, in contrast, resulted in significant reductions in pesticide use in every country where it is grown (Fitt *et al.* 2004) (see chapter 11).

Summary: Impacts of GM crops on pest and weed management
- The results of the Farm Scale Evaluations showing that weed biomass and numbers of some invertebrate groups were reduced under GMHT management in sugar beet and oilseed rape and increased in maize compared with conventional treatments are linked to the weed management of both conventional and GMHT systems. Highly effective weed control practices, such as those chosen for the GMHT crops in the FSE, lead to low numbers of weed seeds and insects. Fewer insects and decreased

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weed seed might reduce the numbers of birds that depend on these insects and seeds as a food source

- The FSE assumed no other changes in field management than GMHT crops replacing non-GM varieties, however, other weed management systems than the one used in the FSE are possible. Use of GMHT technology in the U.S. and in Canada was accompanied by adoption of conservation tillage, which is considered to have beneficial impacts on farmland biodiversity. Conservation tillage results in a greater availability of crop residues and weed seeds improving food supplies for insects, birds, and small mammals.

- Numerous weed species have evolved resistance to a number of herbicides long before the introduction of GMHT crops. The experiences available from regions growing GMHT crops on a large-scale confirm that the development of herbicide-resistances in weeds is not a question of genetic modification, but of the crop- and herbicide management applied by farmers.

- In Canada, no weed species has been observed to demonstrate herbicide tolerance to glyphosate yet. In contrast, the use of glyphosate in continuous cultivation of GMHT soybeans over three years in the U.S. has resulted in the evolution of glyphosate-resistant horseweed.

- Farmers have to add another herbicide to glyphosate to control resistant weed species, but there are alternatives to glyphosate for most weed species that are highly effective and provide good flexibility in application.

- There appear to be more studies that support a small but statistically significant reduction in herbicide use as a result of the adoption of GMHT crops. Because the reduction varies between crops and regions, it is however difficult to draw a general conclusion.

11 Ecological benefits of GM crop cultivation

GM-crops have been adopted for commercial cultivation by farmers in a number of regions over the last decade, as farmers expected potential benefits compared to conventional crops (see chapter 3.3). In order to evaluate the benefits, GM crop cultivation needs to be compared with risks and benefits of the conventional management practices. Conventional crop protection methods relying on chemical pesticides have damaged agricultural land and the environment (see chapter 5). In addition, soil cultivation practices such as tilling have largely contributed to soil degradation by increasing erosion, nutrient loss and degradation of biological processes (Tilman et al. 2002, Ammann 2005). The introduction of GM crops has helped to reduce some environmental impacts of conventional crops and farming methods. This chapter concentrates on actual observed benefits following a decade of commercial cultivation of GM crops distinguishing between direct benefits and those, which are indirectly caused by changes in pest and weed management when adopting GM crops.

11.1 Pesticide reductions due to insect resistant *Bt*-crops

The adoption of *Bt*-maize expressing the insecticidal protein Cry1Ab has resulted in only modest reductions in insecticide applications due to the small area of conventional maize treated with insecticides (see chapter 10.2.1). The results of the large-scale studies performed during the last years provide evidence that *Bt*-maize provides more specific insect control and has fewer side effects on non-target arthropods than most insecticides currently used (see chapter 6.3).

The commercial cultivation of *Bt*-cotton, in contrast, has proven to have resulted both in a significant reduction in the quantity and in the number of insecticide applications (FAO 2004, Fitt *et al.* 2004). Cotton is highly susceptible to several serious insect pests belonging to the budworm-bollworm complex (tobacco budworm, cotton and pink bollworm). These insects constitute a major problem in most cotton-growing areas, because they can cause considerable damage. Conventional cotton cultivation therefore heavily relies on repeated insecticide applications throughout the growing season. Although estimates on pesticide use vary because pesticide use is depending on regional pest pressures, management practices and yearly variations, it appears that the adoption of *Bt*-cotton has significantly reduced the numbers of pesticide applications in every country where *Bt*-cotton has been grown (Tab. 4) (Fitt *et al.* 2004). Moreover, most studies estimate a reduction in the amounts of pesticides used (Phipps & Park 2002, Fitt *et al.* 2004, Brookes & Barfoot 2005). Direct environmental benefits of reduced insecticide applications in *Bt*-cotton resulted in fewer non-target effects (Head *et al.* 2005, Torres & Ruberson 2005) and in reduced pesticide inputs in water (FAO 2004). In China, for example, the number of pesticide applications against lepidopteran pests in cotton has considerably dropped from nine in 1994 to four applications in 2001 following the adoption of *Bt*-cotton (Wu & Guo 2005).

Concerns have been raised that environmental benefits may be lowered by additional spraying against secondary pests that were formerly controlled by the broad spectrum pesticides. There is no published evidence, however, that *Bt*-cotton has resulted in a general change in the pest spectrum leading to an overall increase of pesticide applications (see chapter 6.3.5).

Table 4: Percent reduction of pesticide use in *Bt*-cotton compared to conventional cotton (adapted from Fitt *et al.* 2004)			
Country	**Number of sprays**	**Average pesticide use (%)**	**Reference**
Argentina	- 48	- 49	Qaim *et al.* 2003
USA	- 28		Williams 2003
Australia	- 56	- 43 - 92 [1]	Fitt 2003
India	- 42	- 70	Pyke 2004
China	- 59 - 66	- 70 - 80 - 61	Pray *et al.* 2002 Huang *et al.* 2003 Lu *et al.* 2002
South Africa (small scale)		- 25	Ismael *et al.* 2002
South Africa (large scale)		- 56	Kirsten *et al.* 2002
Mexico	- 54		Traxler *et al.* 2001

[1] Bollgard II

A new *Bt*-cotton variety (Bollgard II) containing two *Bt*-genes (Cry1Ac and Cry2Ab2) was commercially cultivated for the first time in 2003 in Australia and in the United States. Besides its improved performance against the cotton bollworm, Bollgard II confers an additional resistance against secondary pests including soybean and cabbage looper, saltmarsh caterpillar as well as beet and fall armyworm. Data from two years of cultivation in Australia indicate that this new variety has even higher environmental benefits due to the reduction of insecticide use by 92% (Fitt *et al.* 2004) (Tab. 4). The two-gene varieties, which are are expected to replace single *Bt*-gene varieties in the future, can provide better efficacy, reduce or eliminate the necessity for additional chemical pesticides and lower the rate of resistance development.

Pesticide reductions related to the adoption of *Bt*-cotton have also shown to have reduced many immediate as well as longer-term risks to human health (Hossain *et al.* 2004). The use of pesticides is yearly causing considerable poisoning incidences in many, often developing countries. From 1992–1996, for example, there has been an annual average of 54'000 pesticide poisonings of farmers or farm workers in China causing approximately 490 deaths (Hossain *et al.* 2004). As a result of less chemical pesticide spraying in *Bt*-cotton, demonstrable health benefits for farm workers have been documented in China (Pray *et al.* 2002) and South Africa (Bennett *et al.* 2003). Similarly, the adoption of *Bt*-rice in China would prove to have positive impacts on productivity and farmers health. Especially small and poor household farmers would thereby benefit from an 80% reduction in the use of pesticides compared to conventional varieties (Huang *et al.* 2005).

11.2 New weed control strategies offered by GM herbicide tolerant crops

The adoption of GMHT crops has resulted in several weed management changes compared to conventionally managed crops. GMHT crops allow the use of a single broad spectrum herbicide that has a wider spectrum of activity and that may reduce the need for herbicide combinations or chemicals that require multiple applications (Fernandez-

Cornejo & Mc Bride 2002, Brimner *et al.* 2005, Duke 2005). The herbicides used in GMHT crops (glyphosate or glufosinate) are foliar-applied, post-emergence herbicides, which usually allow using herbicides in a more targeted manner. They can be applied after weeds have emerged, i.e. areas with high weed densities can be identified and treated, while areas with low weed pressure can be treated with reduced herbicide amounts. Post-emergence herbicides are thus generally applied at lower rates than soil-applied, pre-emergence herbicides, also because absorption by soil colloids and degradation are reduced (Burnside 1996). Glyphosate and glufosinate are generally considered toxicologically more benign, being less toxic to human health and the environment than many of the herbicides they replace (Fernandez-Cornejo & Mc Bride 2002, Duke 2005). In addition, glyphosate and glufosinate have relatively short soil half-lives and they persist almost half as long in the environment compared to the replaced herbicides. Neither moves readily to ground water, which results in fewer losses of chemicals by leaching and run-off from the field (Duke 2005).

Perhaps the most important environmental benefit of the adoption of GMHT crops is the role they have played in facilitating conservation tillage agriculture (CCOC 2001, Carpenter *et al.* 2002, Fawcett & Towery 2002, Duke 2005). Prior to the introduction of transgenic varieties, most growers used tillage to prepare the soil for planting. Excessive tillage, however, is known to cause soil structure changes, increase the susceptibility to soil erosion and reduce soil moisture. Loss of top soil due to tillage therefore causes environmental damage that can last for centuries. Since the early 1990's, growers have been reducing their tillage operations for soil conservation benefits. The possibility offered by GMHT crops to use broad spectrum herbicides has further encouraged growers to adopt conservation tillage strategies (CCOC 2001, Fawcett & Towery 2002). According to USDA survey data, about 60% of the area planted with GMHT soybean was under conservation tillage in 1997, compared with only about 40% for conventional soybean (Fernandez-Cornejo & Caswell 2006). Because weed control can be done during the post-emergence phase, farmers can use direct-seeding techniques since there is no need for pre-seeding tillage. Conservation tillage leaves a layer of plant residues on the soil surface, preventing soil erosion, reducing evaporation and increasing the ability of the soil to absorb moisture (Fawcett & Towery 2002). A richer soil biota develops that can improve nutrient recycling and this may also help combat crop pests and diseases (Holland 2004). Earthworm populations are generally higher in no-till fields than in conventionally tilled fields (Fawcett & Towery 2002). In addition to a reduction in soil erosion and degradation, less frequent soil cultivation also results in a decrease in the emission of greenhouse gases, partly arising from a reduction in fuel use (Brookes & Barfoot 2005). Gianessi (2005) cites a survey by the American Soybean Association, indicating that U.S. soybean growers reported making fewer tillage passes through their fields since 1995 when GMHT soybean was first introduced. There is also evidence that conservation tillage can provide a wide range of benefits to farmland biodiversity by improving agricultural land as habitat for wildlife. The greater availability of crop residues and weed seeds can improve food supplies for insects, birds, and small mammals (Holland 2004).

Agricultural production systems are complex and diverse. As with the adoption of any new technology, the use of agricultural biotechnology might include positive and possibly less favourable environmental impacts. GM crops systems can help to reduce some environmental risks associated with conventional agriculture, but they will also introduce new challenges that must be addressed. In order to valuate the environmental impacts of GM crop systems, their risks should always be weighed considering their potential benefits and current agricultural practice.

Summary: Ecological benefits of GM crop cultivation

- While the adoption of *Bt*-maize has resulted in only modest reductions in insecticide applications due to the small area of conventional maize treated with insecticides, the commercial cultivation of *Bt*-cotton has proven to have resulted both in a significant reduction in the quantity and in the number of insecticide applications. In addition to direct environmental benefits resulting in fewer non-target effects and in reduced pesticide inputs in water, demonstrable health benefits for farm workers have been documented in several countries due to less chemical pesticide spraying in *Bt*-cotton.

- The adoption of GMHT crops has allowed the use of a single broad spectrum herbicide that may reduce the need for herbicide combinations or chemicals that require multiple applications. The two main herbicides used when growing GMHT crops (glyphosate and glufosinate) are generally less toxic to human health and the environment than many of the herbicides they replace.

- The adoption of GMHT crops has facilitated the change to conservation tillage agriculture. Growers using conservation tillage have reduced their tillage operations, thus preventing soil erosion, soil degradation and runoff of chemicals.

12 Scientific debates on risks of GM crops

The interpretation of collected scientific data is debated controversially by different stakeholders involved in the debate on potential risks of GM crops on biodiversity. Although some groups argue that experiences and solid scientific knowledge are still lacking, the ongoing debate is not primarily due to a lack in scientific data, but more to a lack in clear definitions on how to put a value on effects of GM crops on biodiversity in the context of current agriculture. The interpretation of study results is thereby often challenged by the absence of a baseline for the comparison of effects of GM crops on biodiversity. Consequently, some consider any effect related to GM crops as being undesired, while others correlate it to effects caused by modern agricultural practices recognizing that a multitude of factors involved cause environmental effects.

The interpretation of study results is often challenged by knowledge gaps on the natural variation occurring in any biological system, which is caused by a multitude of factors. Rather than the GM crop alone being the influencing factor, environmental effects are caused by agricultural production systems where the GM crop is one factor among others. Although science can help to assess these natural variations, it will most probably not be possible to elucidate all ecological interactions taking place in such systems. In addition, not every environmental effect is automatically of ecological significance and leading to relevant impacts on biodiversity. In practice, decision-making will thus have to be not purely based on scientific criteria, but will also be strongly influenced by political, social, economical and ethical factors. Ecologically significant effects are only judged unacceptable (i.e. representing a damage) by the society if they are perceived as being linked to a deterioration in quality of a particular entity (e.g. biodiversity). Valuation of scientific data is thus influenced by the individual and subjective perceptions of the terms safety, risk and uncertainty by the society and particularly by the persons involved in decision-making. By performing a risk / benefit assessment comparing positive and negative effects of the GM crop system with current agricultural practice, it is in the end the society's decision whether genetic engineering is considered being safe enough. The following list intends to highlight a number of issues, which are debated controversially in the discussion on the safety of GM crops.

Effects of GM crops on non-target organisms
- There is scientific controversy on the baseline that should be applied when assessing potential effects of insect-resistant GM crops. It is discussed whether this should be the most common agricultural practice used (e.g. pesticides) or a practice that is only used by a few farmers (such as organic farming, which accounts for less than 3% of arable crop production in Switzerland).
- There is a debate to what extent indirect toxic effects, i.e. effects on natural enemies that largely depend on the target pest, should be valuated considering that such effects are common for all pest control methods and not restricted to the use of insect-resistant GM crops.
- It is unclear, which changes in population size and community structures of natural enemies could have an impact on functions and ecosystem services for natural pest regulation.

Impacts of GM crops on soil ecosystems

- A common definition for soil quality has not been found yet.
- Population sizes and community structure of soil microorganism are subject to high variation, and the baseline comparison for ecological implication is still not clear. Standard indicator species have not been defined. Different studies use a range of different parameters and techniques.
- Should influences of plant characteristics (higher/lower lignin content) associated with a particular *Bt*-variety, yet, unrelated to the inserted transgene, be compared to influences caused by plant characteristics of conventional cultivars?
- Is the presence of low percentages of transgenic *Bt*-toxins from *Bt*-crops in soils a reason for concern, considering that *Bt*-toxins are naturally occurring in soils due to the soil bacteria *Bacillus thuringiensis*, and due to *Bt*-spray formulations that are commonly used for insect control in agriculture and forestry?

Gene flow from GM crops to wild relatives

- In most agricultural landscapes, there is usually a gradual transition from peri-agricultural to semi-natural habitats. Although "wild plants" can usually be distinguished from "agricultural weeds", a clear definition of what plant species are considered being truly wild plants is lacking.
- Should effects occurring within agricultural or peri-agricultural environments be given the same importance as those effects, which could occur in natural habitats?
- Should gene flow from GM crops to wild relatives be valuated in a different way than gene flow from conventional crops?

Invasiveness of GM crops into natural habitats

- Is the presence of volunteer GMHT oilseed rape in habitats such as field borders or road verges an unwanted environmental effect, considering that non-transgenic oilseed rape is regularly occurring in such habitats and that HT is not considered to confer a selective advantage in natural habitats?

Impacts of GM crops on pest and weed management and their ecological consequences

- Is it better to have a high biodiversity in-crop (i.e. to have weedy crops), or to enhance off-crop biodiversity (e.g. separate buffer strips outside the fields) providing food for insects and birds?
- There is a need to define criteria for what is considered an agronomic problem and what is regarded being only an agronomic nuisance that could emerge from the cultivation of GMHT crops.
- Should herbicide-resistant weeds that have been caused by GMHT crops be valuated differently than herbicide-resistant weeds that have been caused by unsustainable (non-transgenic) weed management?

Acknowledgements

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Abbreviations

Bt	*Bacillus thuringiensis*
EPA	United States Environmental Protection Agency
ERA	Environmental risk assessment
FSE	Farm Scale Evaluations
GMO	Genetically modified organism
GM	Genetically modified
GMHT	Genetically modified herbicide tolerant
HT	Herbicide tolerance
IR	Insect resistance
OSR	Oilseed rape
SECB	Swiss Expert Committee for Biosafety
USDA	United States Department of Agriculture

Annex

Annex 1
Summary of studies assessing gene flow from oilseed rape to related species.

Gene flow from oilseed rape *(Brassica napus)* to wild turnip *(Brassica rapa)*: Assessment of fitness consequences using hybrids produced by artificial hybridization

Trait / Cultivar	Hybrid generation(s) [1]	Experimental conditions	Method / marker used to confirm hybrid status [2]	Assessed fitness parameters	Hybridization (H) Fitness consequences (F)	Reference
Herbicide-tolerant (HT) oilseed rape (OSR) Glufosinate (Glu)	N (F_1, BC_1) A (BC_2)	Experimental field trial	Herbicide spray, morphology, ploidy level	Pollen viability	H: 42% of the BC_2 plants obtained were Glu-tolerant F: Pollen fertility of BC_1 was greater than 90%	Mikkelsen *et al.* 1996
non-transgenic OSR (cvs. Drakkar, Topas, Westar)	A F_1	Experimental field trial	n.d.	Seed development, survival in the field, pod- and seed set	H: No strong hybridization barrier between *B. napus* and *B. rapa*. F: F_1-hybrids under some conditions nearly as fit as parents	Hauser *et al.* 1998b
non-transgenic OSR (cvs. Topas, Westar)	A F_2, BC_1	Experimental field trial	n.d.	Seed development, survival in the field, pod- and seed set	F: Relatively low average fitness of F_2 and BC_1 as compared to parents	Hauser *et al.* 1998a
HT OSR (Glu)	A BC_3	Growth chamber	PCR, Herbicide spray	Pollen fertility, seed set, survival	F: No significant differences between transgenic and non-transgenic plants in survival and number of seeds per plant. Costs associated with transgene probably negligible	Snow *et al.* 1999
Bt OSR	A BC_1, BC_2	Growth chamber	PCR, Western Blot, ploidy level	n.d.	H: *Bt* trangene was present in hybrids and protein was synthesized at similar levels as corresponding OSR lines F: Not all F_1 lines were able to produce BC_1, but surviving BC_1 were able to produce BC_2	Halfhill *et al.* 2002
HT OSR (Glu)	A F_1	Experimental field trial	Morphology, AFLP, PCR	Flower, pollen and seed production	F: Male fitness among F_1 produced by *B. rapa* is low	Pertl *et al.* 2002
Bt / GFP OSR	A F_1, BC_1, BC_2	Experimental field trial	GFP	Vegetative plant material produced in an insect bioassay	F: No difference found in biomass between BCs and non-transgenic parents under low insect pressure	Mason *et al.* 2003
OSR	A F_1, F_2, sev. BCs	Experimental field trial	n.d.	Seed production	F: Hybrids are not generally less fit than parents. Fitness of both parents and hybrids is strongly frequency-dependent	Hauser *et al.* 2003
Bt / GFP OSR	A F_1	Green house	GFP	Biomass, flower number, seed mass, germination rate	F: Herbivore pressure and plant density had strong impact on relative biomass and on fitness advantages of *Bt*-hybrids over wild type. Greenhouse results can not give a quantitative prediction of *Bt*-spread and persistence in natural habitats	Vacher *et al.* 2004
Bt / GFP OSR	A F_1, BC_1, BC_2	Experimental field trial	GFP	Intraspecific competition with various herbivore pressures and with wheat	F: On average hybrids of various BC generations have lower potential for growth and competitiveness under field conditions than weedy parents	Halfhill *et al.* 2005
Male-sterile OSR	A F_1, BC_1	Growth chamber Experimental field trial	Quantitative PCR	Photosynthetic capability, pollen viability, seed set	H: Expression of transgenes is stable in F_1 hybrids. F: Reproductive fitness of hybrids was significantly lower than in parents, BC_1 had significant lower photosynthetic capability and reproductive fitness than parents. Vegetative vigor of BC_1 is limited.	Ammitzbøll *et al.* 2005

[1] A = Hybrids were produced by artificial hybridization (e.g. hand-pollination)
N = Hybrids formed under natural hybridization conditions
F_1= First filial generation
F_2= Second filial generation
BC_1= First backcross generation etc.

[2] GFP = Green fluorescent protein
PCR = Polymerase chain reaction
AFLP = Amplified fragment length polymorphism

Gene flow from oilseed rape *(Brassica napus)* to wild turnip *(Brassica rapa)*: Assessment of hybridization rates under natural hybridization conditions

Trait / Cultivar	Hybrid generation(s)	Experimental conditions	Method / marker used to confirm hybrid status	Hybridization (H) Fitness consequences (F)	Reference
non-transgenic OSR (cv. Drakkar)	N [1] F_1	Agricultural field [2] (set-aside)	AFLP	H: First study to show introgression between *B. napus* and *B. rapa* under natural condition. Hybrids in weedy natural populations resembled most closely to BC_2 (obtained by controlled crosses)	Hansen *et al.* 2001
Bt OSR	N F_1	Experimental field trial	Antibiotic marker	H: F_1 hybrids have similar levels of expression as crop lines (when hybridization occurs under natural conditions)	Halfhill *et al.* 2002
HT OSR Glyphosate (Gly)	N F_1	Experimental field trial	Herbicide spray, Gly test strip, ploidy level, AFLP	H: Hybridization between *B. napus* and *B. rapa* occurred at approx. 7%	Warwick *et al.* 2003
HT OSR (Gly)	N F_1	Commercial field	Herbicide spray, Gly test strip, ploidy level	H: Hybridization between *B. napus* and *B. rapa* occurred at approx. 13.6%	Warwick *et al.* 2003
GFP OSR	N F_1	Experimental field trial	GFP, morphology, pollen viability, ploidy level	H: Hybridization between *B. napus* and *B. rapa* occurred at approx. 7%	Warwick *et al.* 2003
OSR	N F_1, BC_1	Agricultural field (set-aside)	Chromosome counting, AFLP	H: Introgression progresses primarily with *B. rapa* as maternal plant. Transgenes can be transferred from *B. napus* to *B. rapa*	Hansen *et al.* 2003
Bt / GFP OSR	N F_1, BC_1	Experimental field trial	GFP	H: Hybrids between *B. napus* and *B. rapa* occurred over a wide range of experimental conditions, BC_1 rate was 0.074%	Halfhill *et al.* 2004
HT OSR (Glu)	N F_1	Agricultural field	Herbicide spray, PCR, ploidy level	H: 2 hybrids found in 9500 seedlings	Daniels *et al.* 2005

[1] Hybrids formed under natural pollination conditions
[2] Experimental design corresponds to an agricultural field, but no agricultural cultivation practice was applied in the years before the study (e.g. crop rotation, volunteer management)

Gene flow from oilseed rape *(Brassica napus)* to charlock *(Raphanus raphanistrum)*

Cultivar / trait	Hybrid creation / generation(s)	Experimental conditions	Method / marker used to confirm hybrid status	Fitness parameters used	Hybridization (H) / Fitness consequences (F)	Reference
Male-sterile OSR cv. Brutor	N F_1, F_2, BC_1	Experimental field trial		Seed production	H: Hybrid frequency expected to be at max. 0.2%. Seed production of F_1 = 0.4%, F_2 = 2% F: n.d.	Darmency *et al.* 1998
Non-transgenic OSR (Acetolactat synthase-resistant)	N F_1	Experimental field trial	Morphology, RFLP, ploidy level	Pollen viability	H: No hybrids were detected amongst 25'000 seedlings collected from *R. raphanistrum*. Two hybrids were detected in more than 52 Mio. OSR seedlings. F: Both hybrids had viable pollen and were able to set seed when backcrossed to *R. raphanistrum*, but not OSR	Rieger *et al.* 2001
HT OSR (Glu)	N BC_6	Experimental field trial	Herbicide spray, PCR, ploidy level	Seed production and survival, plant growth and reproduction	H: n.d. F: Fitness level of backcrosses with OSR is 100x lower than of BC with *R. raphanistrum*.	Gueritaine *et al.* 2002
OSR	N F_1	Experimental field trial	Morphology, ploidy level	Seed emergence, flowering time and frequency, diameter of rosette, dry weight	H: n.d. F: F_1 hybrids showed lower seedling emergence, significant delay of emergence and lower survival than both parents	Gueritaine *et al.* 2003
HT OSR (Gly)	A F_1	Greenhouse	Herbicide spray, AFLP, ploidy level	n.d.	H: No hybridization detected F: n.d.	Warwick *et al.* 2003
HT OSR (Gly)	N F_1	Experimental field trial	Herbicide spray	n.d.	H: One hybrid detected in approx. 32'000 seedlings F: n.d.	Warwick *et al.* 2003
HT OSR (Gly)	N F_1	Commercial field	Herbicide spray	n.d.	H: No hybridization detected F: n.d.	Warwick *et al.* 2003
OSR (GFP)	N F_1	Experimental field trial	GFP	n.d.	H: No hybridization detected F: n.d.	Warwick *et al.* 2003
Bt OSR containing GFP	N F_1, BC_1	Experimental field trial	GFP	n.d.	H: No hybridization detected F: n.d.	Halfhill *et al.* 2004

Estimated hybridization rates through gene flow from oilseed rape *(Brassica napus)* to wild mustard *(Sinapis arvensis)* and dog mustard *(Erucastrum gallicum)* [1]

Cultivar / trait	Hybrid creation / generation	Experimental conditions	Method / marker used to confirm hybrid status	Result	Reference
Six non-transgenic OSR cultivars	A / N F_1	Greenhouse Experimental field trial	PCR, Morphology, Southern blot	H: Neither *S. arvensis* nor *B. napus* readiliy hybridise with each other in the greenhouse. Unable to detect gene flow from *B. napus* to *S. arvensis* in the field	Moyes *et al.* 2002
HT OSR (Gly)	N F_1	Commercial field	Herbicide spray	H: No hybridization detected	Warwick *et al.* 2003
HT OSR (Glu)	N F_1	Agricultural field	Herbicide spray, PCR	H: 1 hybrid found in the field	Daniels *et al.* 2005

[1] *E. gallicum* was only investigated in Warwick *et al.* 2003

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ART-Schriftenreihe 1

Ecological impacts of genetically modified crops

The worldwide commercial cultivation of genetically modified (GM) crops has raised concerns about potential adverse effects on the environment, which could result from the use of these crops. Consequently, the risks of GM crops for the environment, and especially for biodiversity, have been extensively assessed before and during their commercial cultivation. Substantial scientific data on environmental effects of the currently commercialized GM crops is available today. Agroscope Reckenholz-Tänikon Research Station ART was commissioned by the Swiss Expert Committee for Biosafety to review this scientific knowledge deriving from the past ten years of worldwide experimental field research and commercial cultivation. The sources of information included peer-reviewed scientific journals, scientific books, reports from countries with extensive GM crop cultivation, as well as reports from international organizations. The data available so far provides no scientific evidence that the commercial cultivation of GM crops has caused environmental harm. Nevertheless, a number of issues related to the interpretation of scientific data on effects of GM crops on the environment are debated controversially. The study highlights these scientific debates and discusses the effects of GM crop cultivation on the environment considering the impacts caused by cultivation practices of modern agricultural systems.

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