Adv Biochem Engin/Biotechnol (2007) 107: 235–278
DOI 10.1007/10_2007_048

Published online: 31 March 2007

Ecological Impacts of Genetically Modified Crops: Ten Years of Field Research and Commercial Cultivation

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Abstract The worldwide commercial cultivation of genetically modified (GM) crops has raised concerns about potential adverse effects on the environment resulting 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 the environmental effects of the currently commercialized GM crops are available today. We have reviewed this scientific knowledge derived from the past 10 years of worldwide experimental field research and commercial cultivation. The review focuses on the currently commercially available GM crops that could be relevant for agriculture in Western and Central Europe (i.e., maize, oilseed rape, and soybean), and on the two main GM traits that are currently commercialized, herbicide tolerance (HT) and insect resistance (IR). The sources of information included peer-reviewed scientific journals, scientific books, reports from regions with extensive GM crop cultivation, as well as reports from international governmental organizations. The data available so far provide no scientific evidence that the cultivation of the presently commercialized 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 present review 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.

Keywords Transgenic crops · Environmental effects · *Bt*-maize · Insect resistance · Herbicide tolerance

Abbreviations

Bt	*Bacillus thuringiensis*
EPA	United States Environmental Protection Agency
FSE	Farm Scale Evaluations
GMO	Genetically modified organism
GM	Genetically modified
GMHT	Genetically modified herbicide tolerant
HT	Herbicide tolerance, herbicide tolerant
IR	Insect resistance, insect resistant
OSR	Oilseed rape
USDA	United States Department of Agriculture

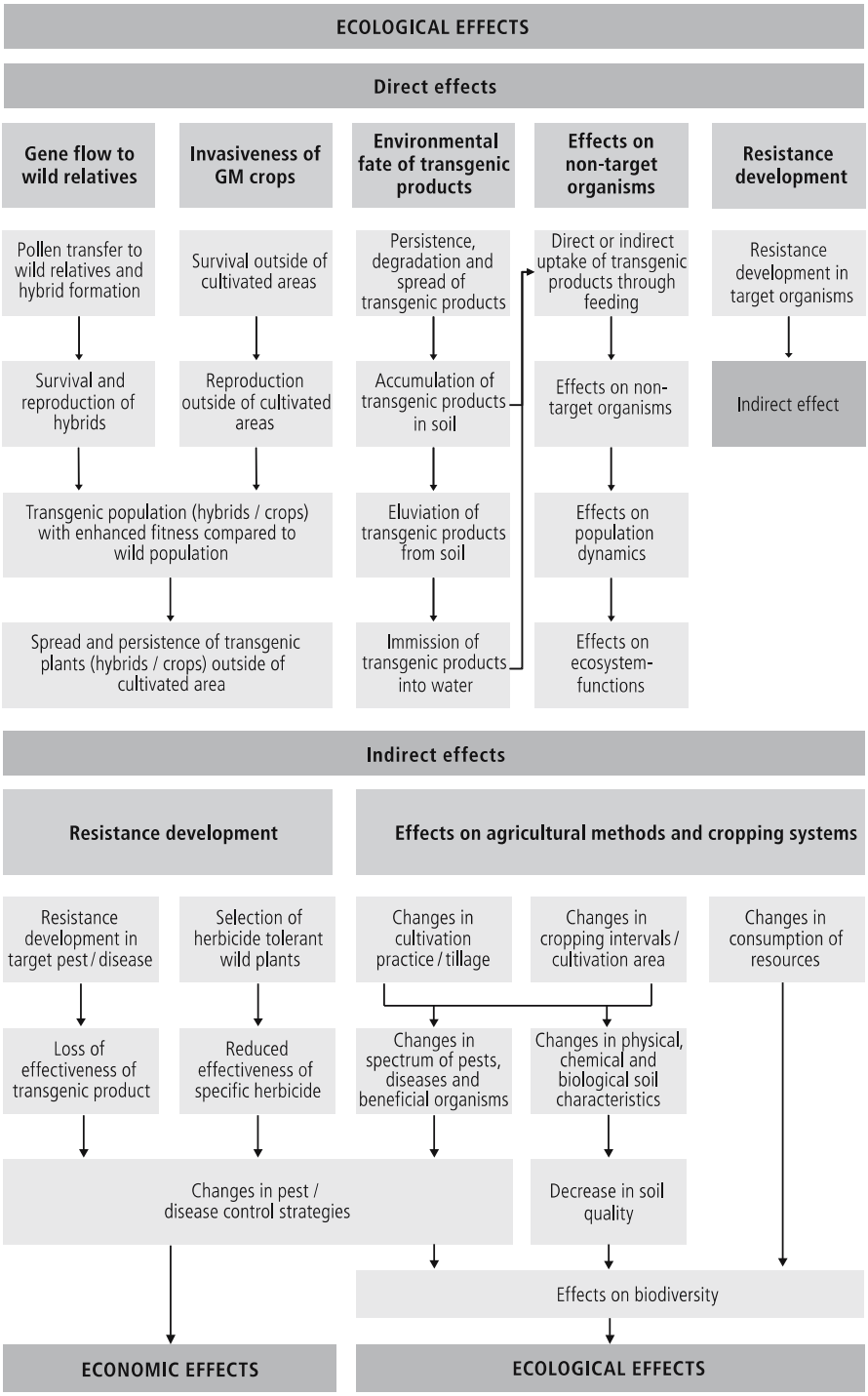
1 Introduction

1.1 GM Crops, Modern Agriculture, and the Environment

The worldwide commercial cultivation of genetically modified (GM) crops has raised concerns about potential adverse effects on the environment from the use of these crops [1–5]. 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 are available. Independent from the use of GM crops, modern agricultural systems have considerable negative impacts on global biodiversity [6–11]. 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 [9, 12]. The negative impact of modern agricultural systems in Europe cannot be ascribed to only one factor, but is 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, for example, was mainly caused by the adoption of high-yielding forage crop varieties, increased fertilizer inputs, frequent applications of herbicides, and the increased purity of crop seed [7, 13]. 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 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 [8, 14, 15]. Potential impacts of GM crops should thus be put in relation to the environmental impacts of modern agricultural practices that took place over the last decades.

1.2 Regulation of GM Crops

Generally, the approval of genetically modified crop varieties is more rigorously regulated than that of conventionally bred crops. Several reasons have lead to this regulation. The protection of human health and the environment is the primary reason for government oversight and regulation. There are other factors besides the safety aspect that have supported government decisions to regulate GM crops. Among others, there is the novelty of transgenic crops, the uncertainty accompanying the transformation process, and pub-


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

lic concerns about the safety of transgenic crops [16]. A thorough pre-market risk assessment of potentially unwanted effects of the GM crop on the environment is thus a prerequisite in obtaining permission to market any GM crop variety. GM crop growing countries generally follow the concepts of familiarity and of substantial equivalence, which state that a GM crop should be compared with its traditional counterpart that has an established history of safe use [17–20]. GM crop varieties that received regulatory approval are considered to present no more risks than comparable conventional varieties with a history of safe use.

1.3 Potential Environmental Effects of GM Crops

Potential environmental effects of the currently commercialized GM crops can roughly be subdivided into direct and indirect effects. 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. 1). GM crops could be able to hybridize with sexually compatible wild relatives and these could subsequently suffer an increased risk 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 pests 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 a number of indirect effects (Fig. 1).

In the present review, the scientific knowledge of the environmental impact of GM crops deriving from 10 years of worldwide experimental field research and commercial cultivation is reviewed. The sources of information included peer-reviewed scientific journals, scientific books, reports from regions with extensive GM crop cultivation, as well as reports from international governmental organizations. The review is focussing on the currently commercially available GM crops that could be relevant for agriculture in Western and Central Europe (i.e., maize, oilseed rape, and soybean), and on the two main GM traits that are currently commercialized, herbicide tolerance (HT) and insect resistance (IR) [21]. Where helpful, experiences gained with other crops such as *Bt*-cotton are considered. GM crops with minor worldwide acreage (e.g., virus-resistant papaya and squash) are not considered. Potential effects

◀ **Fig. 1** Potential direct and indirect effects of genetically modified crops on the environment (adapted from [1, 2])

of GM crops are limited to the environment and to the following main topics: (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, and (5) impacts of GM crops on pest and weed management. In addition, this review identifies the possible ecological benefits that could be derived from the cultivation of GM crops.

2 Effects of *Bt*-Crops on Non-target Organisms

Cry-proteins from *Bacillus thuringiensis* (*Bt*) are by far the most common insecticidal proteins that have been engineered into plants. They represent (up till now) the only insecticidal proteins that are commercially used in GM crops [21]. *Bt cry* genes have been engineered into a large number of plant species such as maize, cotton, potato, tomato, rice, eggplant, and oilseed rape [22–24]. 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 both the Colorado potato beetle and aphids [24]. *Bt*-maize expressing Cry1Ab was initially developed to control a lepidopteran pest, the European Corn Borer (*Ostrinia nubilalis*), but has also shown to be effective against various other lepidopteran pests such as *Sesamia nonagrioides*, *Spodoptera littoralis* and *Helicoverpa zea* [25–27]. *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 [28, 29]. However, due to its recent approval, no experience from commercial cultivation is yet available.

There are concerns that insect-resistant GM crops expressing Cry-proteins from *B. thuringiensis* could harm organisms other than the pest(s) targeted by the toxin. The long-term and wide-scale use of *Bt*-crops over the past 10 years has been accompanied by extensive studies testing potential adverse effects of these crops. One factor of particular interest in this respect is the potential effect of *Bt*-transgenic crops on non-target organisms that provide important ecological and economic services within agricultural systems. This includes parasitoids and predators that are of importance for natural pest regulation, pollinators, and butterflies.

2.1
Effects of *Bt*-crops on Beneficial insects (Predators and Parasitoids)

2.1.1
Lower-Tier Studies in the Laboratory and Greenhouse

The effects of *Bt*-crops on predators have been assessed in a number of studies, most of them using a tritrophic system including a plant, a herbivore and a natural enemy, i.e., predator or parasitoid (reviewed in [30]). Adverse effects on mortality, longevity or development of 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 [31–33]. Results of subsequent studies using several different prey species reared on Cry1Ab-maize, however, showed that the insecticidal protein itself does not directly affect this predator, but that the green lacewing may be affected when feeding on prey species that are susceptible to *Bt*-toxin [34–36]. The negative effect observed was thus entirely prey-quality mediated, i.e., caused by the suboptimal food quality of the lepidopteran larvae used in the experiments. Because lepidopteran larvae are not considered an important prey for *C. carnea* in the field, it is unlikely that *Bt*-maize poses a risk for this predator [36, 37]. Similarly, effects of *Bt*-crops on mortality, development, weight or longevity of hymenopteran parasitoids developing in herbivores reared on transgenic plants were only observed in cases where *Bt*-susceptible herbivores were used as hosts [30]. This is not surprising given that host–parasitoid relationships are usually tight and parasitoids are very sensitive to changes in host quality. The results of the performed lower-tier studies provide evidence that except for the lepidopteran species the toxin is intended for, Cry1Ab does not cause direct toxic effects on any of the arthropod groups examined [30].

2.1.2
Higher-Tier Studies in the Field

More than 50 field experiments, varying greatly in size, duration, and sampling efforts, have been conducted to determine the effects of *Bt*-crops on natural enemies (reviewed in [30]). Most studies assessed the abundance of natural enemies using different methods, while only a few studies compared biological control functions of natural enemies in both *Bt*- and conventional crops. These experimental field studies have only revealed minor, transient or inconsistent effects of *Bt*-crops when compared to a non-*Bt* control [30, 38]. Indirect effects were observed with specialist natural enemies which were virtually absent in *Bt*-fields due to the lack of target pests as prey

or hosts [39, 40]. 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 [41–43]. A 6-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 appear to be ecologically relevant for the biological control function of the natural enemy community [42, 44]. In general, many natural enemies are polyphagous, meaning they are able to switch to other preys in the field when one particular food source is scarce.

The occurrence of indirect effects that are caused by changes in the availability and/or the quality of target herbivores is not restricted to GM technology. Any pest-control measure will cause a reduction in the number of prey and host items, which could consequently affect population densities of natural enemies [30, 45, 46]. Such indirect effects are thus generally not considered to comprise a particular risk of insecticidal GM crops [20, 30].

A number of experimental field studies have included conventional insecticides as a treatment. Since *Bt*-crops are intended to replace or reduce applications of conventional insecticides commonly used in agriculture, insecticide treatments should be considered as one reasonable baseline for a comparative risk assessment [1, 3, 30]. 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 [47–49]; *Bt*-cotton [42, 43, 50–53]). Side effects of more selective insecticides such as indoxacarb (anoxadiazine) or spinosad (amacrolide) largely depended on the spray frequency [49] whereas systemic insecticides (such as imidacloprid, a neonicotinoid) were found to have no or little effect on natural enemies [54]. Although some of the field studies were limited in their spatial scale, and lack 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 [55, 56]. The results of the various studies performed over 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.

2.2 Effects of *Bt*-crops 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. Due to their ecological and economic importance, honey bees are often used as test species in pre-market risk-assessment studies to assess direct toxicity of insecticidal proteins on non-target organisms. Such studies have been conducted for each *Bt*-crop prior to its registration in the United States [57]. Feeding tests with Cry1Ab proteins were conducted on both honey bee larvae and adults and in each case no effects were observed [57]. 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 [46, 58–60]

2.3 Effects of *Bt*-crops on Butterflies

Butterflies are considered as a species group 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*) caused much public interest and led to a debate over the potential risks and the environmental impact of *Bt*-maize. Losey et al. [61] found that when pollen from a commercial variety of *Bt*-maize (event *Bt* 11) 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 4 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. The results of the study drew much attention to (potential) effects of *Bt*-crops on butterflies since the monarch is considered a conservation flagship species in the United States. However, the study also received much criticism and scientists questioned the validity of risk conclusions based on the data obtained in laboratory studies. Later laboratory bioassays showed that the only transgenic *Bt*-maize pollen that consistently affected monarch larvae was pollen from Event 176, an event that has meanwhile been withdrawn from the market. The results suggested that pollen from the most widely planted *Bt*-maize events (MON810 and *Bt* 11) will have no acute effects on larvae in field settings [62, 63] since their pollen expresses 80 times less toxin than Event 176 [63]. The results also suggested that pollen densities used by Losey et al. [61] were in excess compared to pollen densities present in maize fields or that the pollen of event *Bt* 11 used may have been contaminated with non-pollen tissues [64]. Excessive pollen densities of the currently commercialized events (*Bt* 11 and MON810) would be required to obtain relevant adverse effects on larval developments [62].

The critics also felt that in addition to the mere toxicity (hazard), an ecological risk assessment has to consider exposure, i.e., whether the monarch larvae will encounter the *Bt*-toxin and at what level. They also felt that the studies most likely did not address questions like the spatial and temporal overlap of monarch larvae and *Bt*-pollen. Extensive follow-up studies thus determined where the monarchs occur during their breeding season [65], and what percentage of the population of monarchs is possibly affected be the *Bt*-toxin in areas where *Bt*-maize is presently grown [66]. 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 [65]. The proportion of monarch butterfly population exposed to *Bt*-pollen was estimated to be less than 0.8% [66]. Field studies showed that continuous exposure of monarch butterfly larvae to natural deposits of *Bt*-pollen on milkweed plants within maize fields can affect individual larvae, but long-term exposure of larvae to *Bt*-maize pollen throughout their development is detrimental to only a fraction of the breeding population [67]. It was concluded that the risk of exposure is low and that it is unlikely that *Bt*-maize will affect the sustainability of monarch butterfly populations in North America [66, 67]. Furthermore, several authors claimed that effects of *Bt*-maize should be compared to mortality caused by other factors, which is very high in natural monarch butterfly populations, and averages around 80% over the entire larval development period [65, 67]. 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 [57].

3 Effects of *Bt*-crops on Soil Ecosystems

Similar to non-target effects above ground, concerns were raised that *Bt*-crops could have effects on soil organisms and soil functions. The following section discusses the concern that non-target soil organisms and processes could be affected by the accumulation of *Bt*-toxins in soils through the cultivation of the currently commercialized *Bt*-crops.

3.1 Release, Persistence, and Biological Activity of *Bt*-toxins in Soil

Bt-toxins expressed in *Bt*-crops can enter the soil system either via root exudates, via senescent plant material, as well as via damaged and cast-off dead root cells [68–70]. 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 of the transgenic protein in different plant tissues, the progression of decay of the plant cells and the biomass remaining in the field. Expression levels in the *Bt*-maize variety MON810 are estimated to be around 4–7 times higher in leaves than in roots [71].

Persistence of *Bt*-toxins in soil is primarily depending on the protein quantity added and on the rate of inactivation and degradation by biotic and abiotic factors [72]. Degradation rates of *Bt*-toxins are known to be influenced by environmental conditions, soil type, the protein source (purified versus plant-produced) as well as by the particular Cry-protein chosen [45]. 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). The 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 an organism sensitive to the toxin [45].

Persistence, degradation, and inactivation of *Bt*-toxins have been assessed in the laboratory and/or in the field in 11 studies using either *Bt*-maize expressing Cry1Ab, *Bt*-cotton containing other Cry proteins or purified toxins (Table 1). 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 takes place with low amounts (< 2%) that may persist in soil after one season [70]. *Bt*-toxins may partially persist as a consequence of their binding to surface-active clay and humic acid compounds and it seems that bound proteins retain their insecticidal activity [69, 73–76]. 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 [72, 77, 78]. Although estimates on persistence of *Bt*-toxins differ among studies ranging from a few hours [79] to months [70, 80], the results are not essentially conflicting. Much of the described variation can be explained by the fact that the studies employ 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 (Cry1Ab), the crop species (differences in C : N ratio), biotic activity (temperature), soil type (clay content), and the applied crop management practices (no-till with roots remaining in the soil).

Table 1 Summary of results from studies assessing persistence, degradation, and inactivation of *Bt*-toxins in soil

Bt-crop/ *Bt*-toxin	Study conditions	Toxin incorporation into soil	*Bt*-toxin detection	Persistence (days)	Refs.
Cotton tissue/ Cry1Ab and Cry1Ac	Laboratory	Experiments were carried out with field grown cotton tissue/soil/purified toxins in microcosms	Detectable residues (ELISA)[a]	Detection of toxin and insecticidal insecticidal activity at termination of test – 28 d (Cry1Ab) and 56 d (Cry1Ac)	[173]
Microbial toxin and cotton tissue/Cry1Ab and Cry1Ac	Laboratory	Purified toxin and transgenic leaves added to soil in microcosms. Toxins extracted and measured for 140 days	Detectable residues (ELISA)	Initial rapid degradation, low percentage may persist for weeks/ months. Half lives at 22/40 d, depending on clay/organic content of soil	[174]
Maize tissue/ Cry1Ab	Laboratory/ 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%)	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	[175]
Cotton tissue/ Cry2A	Laboratory/ Field; Autumn/ winter MO, USA	Protein incubation in soil for 120 d. Bioassay based on growth inhibition to determine DT50	Bioactivity test	15.5 d (lab) DT50 31.7 d (field) DT50 120 d: down to < 25% (field&Laboratory)	[92]
Maize tissue/ Cry1Ab	Laboratory and field. Includes period of frost	Rhizosphere soil sampled from *Bt*-maize in a plant growth room and in the field	Western blot Bioactivity test	180 d: *Bt*-toxin detectable in rhizosphere soil samples from field (after first frost) around plants that had been dead for several months	[73]
Microbial toxin/Cry1F	Laboratory 25 °C	Mixture of Cry1F pipetted onto soil samples representative of cotton fields	Bioactivity test	< 1 d DT50	[79]
Bt-cotton cultivation/ Cry1Ac	Field ~ 16 °C	Soil samples were collected 3 months after post harvest tillage for 3–6 consecutive years	ELISA Bioactivity test	Not detectable *Bt*-toxins in any of the samples	[77]

Table 1 (continued)

Bt-crop/ *Bt*-toxin	Study conditions	Toxin incorporation into soil	*Bt*-toxin detection	Persistence (days)	Refs.
Maize tissue/ Cry1Ab	Litter bags in field (CH) ~ 9 °C	Leaves (growth chamber) sampled before/after pollen shed, cut&dried and placed in litter bags (5 mm mesh) and buried in soil in mid-October. Monthly analysis.	ELISA Bioactivity test	45 d DT50 145 d DT90 240 d : < 1.5% No degradation in winter (< 5 °C)	[70]
Maize tissue/ Cry1Ab	Soil cages in field (CH) ~ 9 °C	Leaves sampled 3 weeks after pollen shed, cut&dried and added to surface of soil cages (1 mm mesh) with earthworm, tied up in field for 200 d, starting December	ELISA Bioactivity test	35 d DT50 105 d DT90 200 d: 0.3% Degradation continued in winter	[70]
Maize tissue/ Cry1Ab	Laboratory and field. No temperature indication	Laboratory: *Bt*-maize residues added to soil and incubated for 43 days. Field: soil samples from experimental fields after 4 years cultivation of *Bt*-maize	ELISA	Laboratory: 14 d: Cry1Ab not detectable Field: most *Bt*-toxin in subsurface soil at 0–15 cm depth. Not clear if *Bt*-toxin from previous year	[78]
Maize tissue/ Cry1Ab	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	Bioactivity test	No evidence of persistence or accumulation	[72]
Maize tissue/ Cry1Ab	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	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	[68]

[a] ELISA: Enzyme-Linked Immunosorbent Assay
[b] Bioactivity test: sensitive insect bioassay
[c] DT^{50}: Dissipation time 50% = time required for one-half of the initial quantity or concentration to dissipate from a system

3.2 Effects of *Bt*-crops on Soil Microorganisms

To date, the effects of *Bt*-crops on microorganisms have been evaluated in a number of studies which have used a range of different parameters and techniques [81, 82]. 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 [81]. 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 in 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, fertilization, field management, tillage, fungal disease, grassland improvement, nitrification and soil depth [83]. Knowledge of 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 [84]. 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 [81]. These molecular techniques yield fingerprint-type data, which represent an image of the soil microbial community analyzed [82, 85]. An accepted definition of the taxonomic unit, which can be used for defining soil microbial diversity, is, however, clearly lacking [85]. 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 [86].

3.3 Effects of *Bt*-crops on Soil Macroorganisms

Effects of *Bt*-crops on soil macroorganisms have been investigated with nematodes, woodlice, springtails, soil mites and earthworms. Effects of Cry1Ab toxins on nematodes were examined in three studies using soil samples from fields planted with *Bt*-maize and non-*Bt* isolines [86–88]. 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. The authors

concluded that the effects found in *Bt*-maize fall within the normal variation expected in agricultural systems [86].

Three laboratory studies have shown that *Bt*-maize expressing Cry1Ab has no deleterious effects on the woodlice *Porcellio scaber* [89–91]. Wandeler et al. [91] compared six non-*Bt*-maize varieties and two transgenic *Bt*-maize varieties during a 20-day feeding experiment in the laboratory with regards to consumption by *P. scaber*. The consumption of maize leaves differed between the eight maize varieties. While *P. scaber* was found to feed significantly less on one of the two *Bt*-varieties compared to its corresponding non-transgenic control variety, the second transgenic variety was found to be one of the most consumed maize varieties when compared among all eight maize varieties evaluated. These results suggest that consumption by *P. scaber* was more strongly influenced by differences among the maize varieties used than by the factor *Bt*-variety alone.

No negative effects of the *Bt*-toxin Cry1Ab on two springtail species (*Folsomia candida* and *Xenylla grisea*) and on the mite species *Oppia nitens* were found in two laboratory studies [92, 93]. In addition, 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 *F. candida* [57].

Effects of *Bt*-maize expressing Cry1Ab on the earthworm *Lumbricus terrestris* have been studied in the laboratory and under semi-field conditions in two studies [88, 94]. Both studies showed no consistent effects on *L. terrestris*. No significant difference in mortality and in weight of earthworms was found after 40 days in soil planted with *Bt*- or non-*Bt*-maize, or after 45 days in soil amended with the biomass of either *Bt*- or non-*Bt*-maize [88]. Laboratory experiments with adult earthworms feeding on *Bt*- and non-*Bt*-maize litter showed no significant difference in relative weight between the two treatments during the first 160 days of the experiment [94]. After 200 days, the authors found a significant weight loss of 18% of their initial weight when fed on *Bt*-maize litter compared to a weight gain of 4% of the initial weight of non-*Bt*-maize litter-fed earthworms. They concluded that further studies were necessary to see whether or not this difference in relative weight was due to the *Bt*-toxin. Under semi-field conditions, no significant differences in growth patterns were observed in immature *L. terrestris* feeding on *Bt*- and non-*Bt*-litter [94]. Pre-market risk-assessment studies submitted for regulatory approval have not revealed any toxic effect of Cry1A proteins on the earthworm *Eisenia fetida* [57]. In a recent study, the effects of *Bt*-maize on important life-history traits of the widespread earthworm *Aporrectodea caliginosa* were investigated under various experimental conditions [95]. Finely ground *Bt*-maize leaves added to soil had no deleterious effects on survival, growth, development or reproduction in *A. caliginosa*, even in high concentrations that could be considered as a worst-case scenario. Also, growth of juvenile *A. caliginosa* was unaffected when worms were kept in pots with

a growing *Bt*-maize plant. The study confirmed the findings of earlier studies performed with other earthworm species [88, 94]. *Bt*-maize apparently poses minimal risks to earthworms as far as growth and reproduction is concerned.

3.4 The Ecological Significance of Effects of *Bt*-crops on Soil Ecosystems

Neither laboratory nor field studies have shown lethal or sublethal effects of *Bt*-toxins on non-target soil macroorganisms such as earthworms, springtails, soil mites, woodlice or nematodes. For soil microorganisms, many of the studies referred to in this section 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. 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. [86], 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 it difficult to clearly define what is considered an ecologically relevant effect on soil ecosystems. A final conclusion cannot be drawn, however, the scientific data obtained so far suggest that the effects owing to the cultivation of *Bt*-crops fall within the normal variation expected in agricultural systems. These variations 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 [86].

4 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 [3]. 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 [96]. Two major concerns related to transgenes in natural populations will be addressed in this section:

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 (e.g., clover, alfalfa, and grasses). This would then lead to the extinction of the "wild-type" occurring outside arable agriculture in semi-natural habitat-types such as grass- or woodland.

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 [1, 3, 97–99]. 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 review.

4.1 Principles of Gene Flow

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 [99]. 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 fertilization 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 hybridize with members of the recipient population [99, 100]. 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 the various biological factors mentioned, 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 hybridize with a wild relative in some part of their distribution range [100]. 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 hybridize [101]. The potential for gene flow from a specific crop therefore varies from region to region. In the following section, oilseed rape (OSR) (*Brassica napus*) is chosen as an example given that this is currently the only crop where GM varieties are widely commercialized and where gene flow to wild relatives must be considered in Switzerland [102].

4.2 Fitness of Transgenic Hybrids

The key issue whether a weedy plant might evolve to a more competitive weed after hybridization with a related GM crop 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. Fitness consequences of transgenes are therefore essentially depending on the character of the transgenic trait. The presence of a transgene does not in itself appear to be generally beneficial or detrimental in hybrids [96, 98]. 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 insect herbivores [98, 99]. 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 [103, 104]. 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 [98]. 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 environmental conditions, it is difficult to judge how hybrids would behave under natural conditions [98].

4.3 Hybrids of Oilseed Rape Becoming More Competitive Weeds in Agricultural Habitats

Commercial cultivation of oilseed rape (OSR) is to date the only situation that could possibly lead to the introgression of herbicide-tolerant genes into weedy relatives in Western and Central Europe. 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.

Spontaneous hybrids between OSR and *B. rapa* are known to occur under field conditions with either species as the pollen donor [105–110]. However, the transfer of herbicide-tolerant genes from OSR to *B. rapa* seems to vary considerably in agricultural environments (Tables 2, 3). To date, only two studies have discovered herbicide resistant F_1 hybrids between *B. rapa* and OSR under commercial agricultural cultivation conditions [105, 110]. In a Canadian study conducted in Quebec, 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 [110]. 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-m strip next to the crop edge had been sampled, and only two out of approximately 9500 seedlings were found to have incorporated the herbicide-tolerant gene [105]. 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*

The probability of gene flow from OSR to *S. arvensis* [111] and *R. raphanistrum* [112–114] seems to be very low (Tables 4, 5). The occurrence of spontaneous hybrids in commercial fields is therefore unlikely [105, 110].

4.4
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 taxa has been observed [96, 98, 99]. Hybridization-mediated environmental impacts from the currently commercialized GM crops seem not to be any different from those of traditionally bred crops. However, transgene escape into wild populations of creeping bentgrass (*Agrostis stolonifera*) from experimental fields of GMHT creeping bentgrass has recently been demonstrated in the U.S. [115]. The long-term fate and ecological impacts of these transgenes within wild *A. stolonifera* pop-

Table 2 Summary of studies assessing 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)	Experimental conditions	Method/marker used to confirm hybrid status[b]	Assessed fitness parameters	Hybridization (H) Fitness consequences (F)	Refs.
Herbicide-tolerant (HT) Oilseed rape (OSR) Glufosinate (Glu)	(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%	[176]
Non-transgenic OSR (cvs. Drakkar, Topas, Westar)	F_1	Experimental field trial	n.d.	Seed development, survival in the field, pod- and seet set	H: No strong hybridization barrier between *B. napus* and *B. rapa*. F: F_1-hybrids under some conditions nearly as fit as parents	[177]
Non-transgenic OSR (cvs. Topas, Westar)	F_2, BC_1	Experimental field trial	n.d.	Seed development, survival in the field, pod- and seet set	F: Relatively low average fitness of F_2 and BC_1 as compared to parents	[178]
HT OSR (Glu)	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	[179]

Table 2 (continued)

Trait/Cultivar	Hybrid generation(s)	Experimental conditions	Method/marker used to confirm hybrid status[b]	Assessed fitness parameters	Hybridization (H) Fitness consequences (F)	Refs.
Bt OSR	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	[106]
HT OSR (Glu)	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	[180]
Bt/GFP OSR	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	[103]
OSR	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	[181]

Table 2 (continued)

Trait/Cultivar	Hybrid generation(s)	Experimental conditions	Method/marker used to confirm hybrid status[b]	Assessed fitness parameters	Hybridization (H) Fitness consequences (F)	Refs.
Bt/GFP OSR	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 cannot give a quantitative prediction of *Bt*-spread and persistence in natural habitats	[104]
Bt/GFP OSR	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	[182]
Male-sterile OSR	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 of BC_1 is limited.	[183]

[a] Hybrids of F_1 and BC_1 generations used in this study were produced under natural hybridization conditions;
F_1= first filial generation, F_2= second filial generation, BC_1= first backcross generation etc.
[b] *GFP* green fluorescent protein; *PCR* Polymerase chain reaction; *AFLP* Amplified fragment length polymorphism; *RFLP* Restriction fragment length polymorphism; *n.d.* not determined

Table 3 Summary of studies assessing 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)[a]	Experimental conditions	Method/marker used to confirm hybrid status[b]		Hybridization (H) Fitness consequences (F)	Refs.
Non-transgenic oilseed rape (OSR) (cv. Drakkar)	F_1	Agricultural field (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)	[108]
Bt OSR	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)	[106]
Herbicide-tolerant (HT) OSR Glyphosate (Gly)	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%	[110]
HT OSR (Gly)	F_1	Commercial field	Herbicide spray, Gly test strip, ploidy level	H:	Hybridization between *B. napus* and *B. rapa* occurred at approx. 13.6%	[110]
GFP OSR	F_1	Experimental field trial	GFP, morphology, pollen viability ploidy level	H:	Hybridization between *B. napus* and *B. rapa* occurred at approx. 7%	[110]
OSR	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*	[109]
Bt/GFP OSR	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%	[107]
HT OSR (Glu)	F_1	Agricultural field	Herbicide spray, PCR, ploidy level	H:	2 hybrids found in 9500 seedlings	[105]

[a,b] Abbreviations: see Table 2

Table 4 Summary of studies assessing gene flow from oilseed rape (*Brassica napus*) to charlock (*Raphanus raphanistrum*)

Trait/Cultivar	Hybrid creation/ generation(s) [a]	Experimental conditions	Method/marker used to confirm hybrid status [b]	Fitness parameters used	Hybridization (H) Fitness consequences (F)	Refs.
Male-sterile oilseed rape (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%	[112]
Non-transgenic OSR (Acetolactat synthase-resistant)	N F_1	Experimental field trial	Morphology, RFLP, ploidy level	Pollen viability	H: No hybrids were detected amongst 25000 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	[184]
Herbicide-tolerant (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 100× lower than of BC with *R. raphanistrum*.	[114]
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	[113]

Table 4 (continued)

Trait/Cultivar	Hybrid creation/ generation(s) [a]	Experimental conditions	Method/marker used to confirm hybrid status [b]	Fitness parameters used	Hybridization (H) Fitness consequences (F)	Refs.
HT OSR (Gly)	A F_1	Green house	Herbicide spray, AFLP, ploidy level	n.d.	H: No hybridization detected F: n.d.	[110]
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.	[110]
HT OSR (Gly)	N F_1	Commercial field	Herbicide spray	n.d.	H: No hybridization detected F: n.d.	[110]
OSR (GFP)	N F_1	Experimental field trial	GFP	n.d.	H: No hybridization detected F: n.d.	[110]
Bt-OSR containing GFP	N F_1, BC_1	Experimental field trial	GFP	n.d.	H: No hybridization detected F: n.d.	[107]

[a] A = Hybrids were produced by artificial hybridization (e.g. hand-pollination),
N = Hybrids produced under natural hybridization conditions

[b] Abbreviations: see Table 2

Table 5 Summary of studies assessing hybridization rates between oilseed rape (*Brassica napus*) and wild mustard (*Sinapis arvensis*) and dog mustard (*Erucastrum gallicum*) [a]

Trait/Cultivar	Hybrid creation/ generation [b]	Experimental conditions	Method/marker used to confirm hybrid status [c]		Result	Refs.
Six non-transgenic oilseed rape (OSR) cultivars	A/N F_1	Green house 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	[111]
Herbicide-tolerant (HT) OSR (Gly)	N F_1	Commercial field	Herbicide spray	H:	No hybridization detected	[110]
HT OSR (Glu)	N F_1	Agricultural field	Herbicide spray, PCR	H:	1 hybrid found in the field	[105]

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

[b] A = Hybrids were produced by artificial hybridization (e.g., hand-pollination), N = hybrids produced under natural hybridization conditions

[c] Abbreviations: see Table 2

ulations remain to be determined. Gene flow from traditional crops has on some occasions created problems by bringing wild relatives closer to extinction. There are 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 [96]. 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 [116].

4.5 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 in the order of what would be expected for non-transgenic crops [96]. Much empirical information about crop-wild relative hybridization is now available [97] indicating that such hybridization occurs when sexually compatible wild relatives are present in close proximity to the crop, albeit at low (and variable) rates [99]. Hybridization between conventional (non-GM) crops and their wild relatives has occasionally caused problems in ecological and evolutionary time. 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 [98]. 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.

5 Invasiveness of GM Crops into Natural Habitats

The awareness of the problems that sometimes accompanied the deliberate or accidental introduction of non-native species into new environments has a long history [117]. 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 [118–120]. Although the great majority of accidental introductions undoubtedly failed to become established, a substantial number became established, and some of these became serious pests [121]. 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 [121].

5.1 Multiple Herbicide Resistances in Oilseed Rape 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 herbicide-tolerant OSR commonly grown in Canada: glyphosate (counting for 59% of the total acreage in 2001) and glufosinate-resistant varieties (16%)—both obtained by genetic engineering—as well as a non-transgenic imidazolinone-resistant type (25%) [122]. 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 3 years of commercial cultivation of GMHT OSR, two triple-herbicide resistant volunteers were reported at a field site in western Canada [123] and a study at 11 sites in Saskatchewan, Canada, reported double-resistant OSR volunteers [124]. 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 [122, 123, 125]. 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 [125]. However, there is no evidence at present that the extensive cultivation of GMHT OSR over several years in western Canada has resulted in an increase of volunteer OSR that would have been caused by the herbicide-tolerant traits [126]. 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 [123, 126]. 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 [122]. In all crops, except field peas, alternative herbicides are able to control herbicide-tolerant OSR because glyphosate and glufosinate are not used in crops other than OSR at this time in western Canada [126]. Although not all volunteer OSR are killed by the herbicide application, most survivors are affected by the combination of crop competition and partial herbicide control that reduces seed set. Furthermore, there are a multitude of cultural and mechanical practices that are recommended to growers to manage multiple-GMHT OSR volunteers. These

include [122] (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.

5.2 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 12 natural habitats and under a range of climatic conditions [127]. 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 12 different habitats and monitored their performance over a period of 10 years [128]. 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.

5.3 Conclusions on the Invasiveness of GM Crops Into Natural Habitats

Despite the extensive commercial cultivation of GMHT OSR in western Canada for several years, there is currently no evidence 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 [126]. De-domestication 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 [129]. Globally, the feral plants that cause much of the economic damage are imported horticultural plants [118–120]. Unlike annual crops, these horticultural plants are mostly perennials that have extensive sexual and asexual reproduction.

6
Weed Management Changes Related to GM Herbicide-tolerant Crops

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 Sect. 1.1). 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 have been perceived more strongly and have been judged to be more important since the adoption of GM crops and that these concerns were less prevalent in the past.

6.1
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 [130, 131], 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 [132–134].

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. 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 [135]. 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 [136]. 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 weed and insect abundance.

The results of the FSE led some to the rather simplistic conclusion that the use of GMHT crops generally leads to lower weed and insect densities, which consequently affect farmland biodiversity, and especially bird populations. Although the FSE were one of the most extensive ecological studies ever conducted, they were not without limitations [137, 138]. As the authors of the

FSE studies 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" [139]. There are two important limits that we feel 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. Taking into account that all three crops occupied less than 15% of the total arable field surface of Great Britain in any year [135], 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 [8]. 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 generalists [8]. 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 [135]. The results are therefore linked to the weed-management system practiced 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 [137]. 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 [140, 141] (see Sect. 7). 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 [142]. Similarly, studies conducted in the UK have shown that alternative scenarios to those resulting from the FSE are possible for GMHT sugar beet [132, 134]. 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 autumn bird

food availability, or provide early season benefits to invertebrates and nesting birds [134].

6.2 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 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 [143, 144]. The commercialization 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 [143, 144]. 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 [145, 146]. After almost three decades of glyphosate use, tolerance to glyphosate has only been reported in eight weed species worldwide [143].

The experiences available from regions growing GMHT crops on a large-scale confirm that the development of herbicide-resistance in weeds is not a question of genetic modification, but of the herbicide management applied by farmers. In Canada, no weed species have been observed yet that demonstrated herbicide tolerance to glyphosate [146]. Although no long-term studies have been conducted, no significant shifts in weed populations and no major difficulties in the management of weeds in agricultural settings have been attributed to the widespread cultivation of GMHT crops 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 widespread 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 consequence, within 3 years after the introduction of GMHT soybean varieties, glyphosate-resistant horseweed (*Conyza canadensis*) was detected [147]. 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 selection pressure on the weed community.

Glyphosate-resistant weeds have been described by some as "super weeds", and there have even been inferences that glyphosate-resistant weed presence could reduce farmland value. Although farmers have to add another herbicide to glyphosate to control the resistant weed species, there are alternatives to glyphosate that are highly effective and provide good flexibility in application timing for most weed species. There is, however, no question that glyphosate-resistant weeds will increase the costs of weed management to 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 application flexibility would present a greater cost to many farmers than the additional herbicide 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 [148–150].

6.3
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 [151, 152], but there appear to be more studies that support a small but statistically significant reduction in herbicide use [140, 153–155]. Because the reduction varies between crops and regions, it is 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 [153]. The average soybean herbicide application rates in the U.S., in contrast, have slightly increased by 3% since the introduction of GMHT soybean (in terms of active ingredients per acreage) [140, 155]. It would, however, be insufficient to assess herbicide use only by comparing the quantities of herbicides applied, even if expressed as 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 less toxic to humans and to the environment than the previously used [155, 156]. 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 [154, 157, 158]. 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 Sect. 7).

7
Possible Ecological Benefits of GM Crop Cultivation

7.1
Pesticide Reductions due to Insect-resistant 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 incidence or where the development of resistant pests impedes the use of pesticides [159, 160]. The benefits related to the adoption of *Bt*-crops may comprise both higher yields and significant reductions in pesticide use for some crops. While 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, 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 [159, 161]. Cotton is highly susceptible to several serious insect pests belonging to the budworm-bollworm complex, i.e., tobacco budworm (*Heliothis virescens*), cotton bollworm (*Helicoverpa* spp.) and pink bollworm (*Pectinophora gossypiella*). These insects constitute a major problem in most cotton-growing areas because they can cause considerable damage. Conventional cotton cultivation therefore relies heavily 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 [161]. Moreover, most studies estimate a reduction in the amount of pesticides used [141, 154, 161]. Direct environmental benefits of reduced insecticide applications in *Bt*-cotton resulted in fewer non-target effects [55, 56] and in reduced pesticide inputs

in water [159]. 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 [162]. Concerns have been raised that these environmental benefits may be lowered by additional spraying against secondary pests that were formerly controlled by the broad spectrum pesticides. There is, however, no published evidence that *Bt*-cotton has resulted in a general change in the pest spectrum leading to an overall increase of pesticide applications. In addition to direct environmental benefits, 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 [163–166].

7.2 New Weed Control Strategies Offered by GM Herbicide-Tolerant Crops

The adoption of GMHT crop varieties has resulted in several weed management changes compared to conventionally managed crops. GMHT crop varieties 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 [153, 155, 156]. 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 [167]. Glyphosate and glufosinate are considered being less toxic to human health and the environment than many of the herbicides they replace [155, 156]. Both 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 [156].

Perhaps the most important environmental benefit of the adoption of GMHT crops is the possibility to use broad spectrum herbicides, which encouraged growers to adopt conservation tillage strategies [140, 156, 168, 169]. Prior to the introduction of transgenic HT crop 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 topsoil due to tillage therefore causes environmental damage that can last for centuries. Since the early 1990s, growers have been reducing their tillage operations for soil conservation benefits. 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 [170]. Gianessi [171] 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. 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 [169]. A richer soil biota develops that can improve nutrient recycling and this may also help combat crop pests and diseases [142]. Earthworm populations are generally higher in no-till fields than in conventionally tilled fields [169]. 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 [154]. 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 [142].

8 Scientific Debates on the Ecological Impact of GM Crops

The interpretation of collected scientific data is debated controversially by different stakeholders involved in the debate on potential impact of GM crops on biodiversity. Although some groups argue that experience and solid scientific knowledge are still lacking, the ongoing debate is generally not purely due to a lack of scientific data, but more to an ambiguous interpretation 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 evaluation of environmental effects of GM crops. Consequently, some consider any effect related to GM crops as being undesired, while others compare it to effects caused by modern agricultural practices recognizing that a multitude of factors involved cause environmental effects. The interpretation of study results is further often challenged by knowledge gaps on the natural variation occurring in any biological system. 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 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. The following list intends to highlight a number of issues, which mainly in Europe are currently 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., integrated pest management), a practice like organic farming, which is only practiced by a low number of farmers, or a (hypothetical) practice that may represent the optimal system for the environment.
- 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.

Impacts of GM Crops on Soil Ecosystems

- A commonly accepted definition for soil quality has not yet been found.
- 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.
- Is the presence of low percentages of activated transgenic *Bt*-toxin(s) 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 that *Bt*-spray formulations 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 to wild relatives?

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?
- Should herbicide-resistant weeds that have been caused by GMHT crops be valuated differently than herbicide-resistant weeds that have been caused by conventional (non-transgenic) weed management?

9 Conclusions

The risks of GM crops for the environment, and especially for biodiversity, have been extensively assessed worldwide over the past 10 years of commercial cultivation of GM crops. Consequently, substantial scientific data on environmental effects of the currently commercialized GM crops are available today, and will further be obtained given that several research programmes are underway in a number of countries. The data available so far provide no scientific evidence that the commercial cultivation of GM crops has caused environmental impacts beyond the impacts that have been caused by conventional agricultural management practices. 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 scientific data are 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"). 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 to represent a relevant environmental impact.

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 favorable environmental impacts. GM cropping systems can help to reduce some environmental impacts associated with conventional agriculture, but they will also introduce new challenges that must be addressed. When discussing the risks of GM crops, one has to rec-

ognize 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 [172]. We thus believe that both benefits and risks of GM crop systems should be compared with those of current agricultural practices.

Acknowledgements We would like to thank the Swiss Expert Committee for Biosafety for major funding of this review. We further thank Michèle Stark for help on an early draft of the manuscript.

References

1. Dale PJ, Clarke B, Fontes EMG (2002) Nat Biotechnol 20:567
2. Wolfenbarger LL, Phifer P (2000) Science 290:2088
3. Conner AJ, Glare TR, Nap J-P (2003) Plant J 33:19
4. Pretty J (2001) Environ Conserv 28:248
5. Snow AA, Andow DA, Gepts P, Hallerman EM, Power A, Tiedje JM, Wolfenbarger LL (2005) Ecol Appl 15:377
6. Stoate C, Boatman ND, Borralho RJ, Carvalho CR, de Snoo GR, Eden P (2001) J Environ Manage 63:337
7. Hails RS (2002) Nature 418:685
8. Robinson RA, Sutherland WJ (2002) J Appl Ecol 39:157
9. Chapin FS, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Diaz S (2000) Nature 405:234
10. Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Nature 418:671
11. Ammann K (2005) Trend Biotechnol 23:388
12. McLaughlin A, Mineau P (1995) Agric Ecosyst Environ 55:201
13. Walter T, Buholzer S, Kühne A, Schneider K (2005) In: Herzog F, Walter T (eds) Evaluation der Ökomassnahmen Bereich Biodiversität. Agroscope FAL Reckenholz. Eidgenössische Forschungsanstalt für Agrarökologie und Landbau, Zürich (Schriftenreihe der FAL Nr. 56)
14. Chamberlain DE, Fuller RJ, Bunce RGH, Duckworth JC, Shrubb M (2000) J Appl Ecol 37:771
15. Royal Society (2003) GM crops, modern agriculture and the environment. The Royal Society, London, p 17
16. Jaffe G (2004) Transgen Res 13:5
17. CFIA (2004) Canadian Food Inspection Agency, Ottawa, p 33
18. EFSA (2004) Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. European Food Safety Authority, Brussels, p 94
19. EPA (1998) Guidelines for ecological risk assessment. US Environ Protection Agency, Washington, DC, p 80
20. OECD (1993) Safety considerations for biotechnology: scale-up of crop plants. Organisation for Economic Co-Operation and Development, Paris, p 43

21. James C (2005) Global status of commercialized biotech/GM crops: 2005, ISAAA Brief No. 34. International Service for the Acquisition of Agri-biotech Applications, Ithaca, NY, p 11
22. de Maagd RA (2004) In: Nap JPH, Atanassov A, Stiekema WJ (eds) Genomics for biosafety in plant biotechnology. IOS Press, Amsterdam, p 117
23. Ely S (1993) In: Entwistle PF, Cory JS, Bailey MJ, Higgs S (eds) *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. Wiley, Chichester, p 105
24. Shelton AM, Zhao JZ, Roush RT (2002) Ann Rev Entomol 47:845
25. Pilcher CD, Rice ME, Obrycki JJ, Lewis LC (1997) J Econ Entomol 90:669
26. Gonzales-Nunez M, Ortego F, Castanera P (2000) J Econ Entomol 93:459
27. Dutton A, Romeis J, Bigler F (2005) Entomol Experiment Appl 114:161
28. AGBIOS (2006) Biotech Crop Database. AGBIOS, Merrickville, Ontario www.agbios.com
29. Ward DP, de Gooyer TA, Vaughn TT, Head G, McKee MJ, Astwood JD, Pershing JC (2005) In: Vidal S, Kuhlmann U, Edwards CR (eds) Western corn rootworm: ecology and management. CABI Publishing, Wallingford UK, p 239
30. Romeis J, Meissle M, Bigler F (2006) Nat Biotechnol 24:63
31. Hilbeck A, Baumgartner M, Fried PM, Bigler F (1998) Environ Entomol 27:480
32. Hilbeck A, Moar WJ, Pusztai-Carey M, Filippini A, Bigler F (1998) Environ Entomol 27:1255
33. Hilbeck A, Moar WJ, Pusztai-Carey M, Filippini A, Bigler F (1999) Entomol Experiment Appl 91:305
34. Dutton A, Klein H, Romeis J, Bigler F (2002) Ecol Entomol 27:441
35. Rodrigo-Simon A, De Maagd RA, Avilla C, Bakker PL, Molthoff J, Gonzalez-Zamora JE, Ferre J (2006) Appl Environ Microbiol 72:1595
36. Romeis J, Dutton A, Bigler F (2004) J Insect Physiol 50:175
37. Dutton A, Romeis J, Bigler F (2003) Biocontrol 48:611
38. Eizaguirre M, Albajes R, Lopez C, Eras J, Lumbierres B, Pons X (2006) Transgen Res 15:1
39. Pilcher CD, Rice ME, Obrycki JJ (2005) Environ Entomol 34:1302
40. Riddick EW, Dively G, Barbosa P (1998) Ann Entomol Soc USA 91:647
41. Daly T, Buntin GD (2005) Environ Entomol 34:1292
42. Naranjo SE (2005) Environ Entomol 34:1193
43. Whitehouse MEA, Wilson LJ, Fitt GP (2005) Environ Entomol 34:1224
44. Naranjo SE, Head G, Dively GP (2005) Environ Entomol 34:1178
45. Clark BW, Phillips TA, Coats JR (2005) J Agric Food Chem 53:4643
46. O'Callaghan M, Glare TR, Burgess EPJ, Malone LA (2005) Ann Rev Entomol 50:271
47. Candolfi MP, Brown K, Grimm C, Reber B, Schmidli H (2004) Biocontrol Sci Technol 14:129
48. Meissle M, Lang A (2005) Agric Ecosyst Environ 107:359
49. Musser FR, Shelton AM (2003) J Econ Entomol 96:71
50. Men XY, Ge F, Edwards CA, Yardim EN (2004) Phytoparasitica 32:246
51. Bambawale OM, Singh A, Sharma OP, Bhosle BB, Lavekar RC, Dhandapani A, Kanwar V, Tanwar RK, Rathod KS, Patange NR, Pawar VM (2004) Curr Sci 86:1628
52. Hagerty AM, Kilpatrick AL, Turnipseed SG, Sullivan MJ, Bridges WC (2005) Environ Entomol 34:105
53. Wu KM, Guo YY (2003) Environ Entomol 32:312
54. de la Poza M, Pons X, Farinos GP, Lopez C, Ortego F, Eizaguirre M, Castanera P, Albajes R (2005) Crop Protect 24:677

55. Head G, Moar M, Eubanks M, Freeman B, Ruberson J, Hagerty A, Turnipseed S (2005) Environ Entomol 34:1257
56. Torres JB, Ruberson JR (2005) Environ Entomol 34:1242
57. EPA (2001) Biopesticides registration action document – *Bacillus thuringiensis* plant-incorporated protectants. US Environmental Protection Agency, Washington DC, p 481
58. Babendreier D, Kalberer NM, Romeis J, Fluri P, Mulligan E, Bigler F (2005) Apidologie 36:585
59. Malone LA (2004) Bee World 85:29
60. Malone LA, Pham-Delegue MH (2001) Apidologie 32:287
61. Losey JE, Rayor LS, Carter ME (1999) Nature 399:214
62. Hellmich RL, Siegfried BD, Sears MK, Stanley-Horn DE, Daniels MJ, Mattila HR, Spencer T, Bidne KG, Lewis LC (2001) Proc Natl Acad Sci USA 98:11925
63. Stanley-Horn DE, Dively GP, Hellmich RL, Mattila HR, Sears MK, Rose R, Jesse LCH, Losey JE, Obrycki JJ, Lewis L (2001) Proc Natl Acad Sci USA 98:11931
64. Anderson PL, Hellmich RL, Sears MK, Sumerford DV, Lewis LC (2004) Environ Entomol 33:1109
65. Oberhauser KS, Prysby MD, Mattila HR, Stanley-Horn DE, Sears MK, Dively G, Olson E, Pleasants JM, Lam WKF, Hellmich RL (2001) Proc Natl Acad Sci USA 98: 11913
66. Sears MK, Hellmich RL, Stanley-Horn DE, Oberhauser KS, Pleasants JM, Mattila HR, Siegfried BD, Dively GP (2001) Proc Natl Acad Sci USA 98:11937
67. Dively GP, Rose R, Sears MK, Hellmich RL, Stanley-Horn DE, Calvin DD, Russo JM, Anderson PL (2004) Environ Entomol 33:1116
68. Baumgarte S, Tebbe CC (2005) Molec Ecol 14:2539
69. Saxena D, Flores S, Stotzky G (1999) Nature 402:480
70. Zwahlen C, Hilbeck A, Gugerli P, Nentwig W (2003) Molec Ecol 12:765
71. Mendelsohn M, Kough J, Vaituzis Z, Matthews K (2003) Nat Biotechnol 21:1003
72. Dubelman S, Ayden BR, Bader BM, Brown CR, Jiang CJ, Vlachos D (2005) Environ Entomol 34:915
73. Saxena D, Stotzky G (2000) FEMS Microbiol Ecol 33:35
74. Stotzky G (2004) Plant Soil 266:77
75. Tapp H, Stotzky G (1998) Soil Biol Biochem 30:471
76. Venkateswerlu G, Stotzky G (1992) Curr Microbiol 25:225
77. Head G, Surber JB, Watson JA, Martin JW, Duan JJ (2002) Environ Entomol 31:30
78. Hopkins DW, Gregorich EG (2003) Eur J Soil Sci 54:793
79. Herman RA, Evans SL, Shanahan DM, Mihaliak CA, Bormett GA, Young DL, Buehrer J (2001) Environ Entomol 30:642
80. Sims SR, Ream JE (1997) J Agric Food Chem 45:1502
81. Bruinsma M, Kowalchuk GA, van Veen JA (2003) Biol Fert Soils 37:329
82. Widmer F (2007) Assessing Effects of Transgenic Crops on Soie Microbial Communities (in this volume). Springer, Heidelberg
83. Cartwright C, Lilley A, Kirton J (2004) Mechanisms for investigating changes in soil ecology due to GMO releases. DEFRA Department for Environment Food and Rural Affairs, London, p 133
84. Motavalli PP, Kremer RJ, Fang M, Means NE (2004) J Environ Qual 33:816
85. Widmer F, Oberholzer HR (2003) In: Francaviglia R (ed) Agricultural impacts on soil erosion and soil biodiversity: developing indicators for policy analysis. OECD Organisation for Economic Co-operation and Development, Rome, Italy, p 551

86. Griffiths BS, Caul S, Thompson J, Birch ANE, Scrimgeour C, Andersen MN, Cortet J, Messean A, Sausse C, Lacroix B, Krogh PH (2005) Plant Soil 275:135
87. Manachini B, Lozzia GC (2002) Boll Zool Agr Bachic Ser II 34:85
88. Saxena D, Stotzky G (2001) Soil Biol Biochem 33:1225
89. Escher N, Käch B, Nentwig W (2000) Basic Appl Ecol 1:161
90. Pont B, Nentwig W (2005) Biocontr Sci Technol 15:341
91. Wandeler H, Bahylova J, Nentwig W (2002) Basic Appl Ecol 3:357
92. Sims SR, Martin JW (1997) Pedobiologia 41:412
93. Yu L, Berry RE, Croft BA (1997) J Econ Entomol 90:113
94. Zwahlen C, Hilbeck A, Howald R, Nentwig W (2003) Molec Ecol 12:1077
95. Vercesi ML, Krogh PH, Holmstrup M (2006) Appl Soil Ecol 32:180
96. Ellstrand NC (2003) Dangerous liaisons? When cultivated plants mate with their wild relatives. The John Hopkins University Press, Baltimore
97. de Nijs HCM, Bartsch D, Sweet JB (2004) Introgression from genetically modified plants into wild relatives. CABI Publishing, Wallingford UK
98. Hails RS, Morley K (2005) Trend Ecol Evolut 20:245
99. Stewart CN, Halfhill MD, Warwick SI (2003) Nat Rev Genet 4:806
100. Ellstrand NC, Prentice HC, Hancock JF (1999) Ann Rev Ecol Syst 30:539
101. GM Sci Review Panel (2003) GM science review: first report. Department of Trade and Industry, London, p 296
102. Jacot Y, Ammann K (1999) In: Ammann K, Jacot Y, Simonsen V, Kjellson G (eds) Methods for Risk Assessment of Transgenic Plants III. Ecological risks and prospects of transgenic plants, vol 3. Birkhäuser Verlag, Basel, p 99
103. Mason P, Braun L, Warwick SI, Zhu B, Stewart CN (2003) Environ Biosafe Res 2:263
104. Vacher C, Weis AE, Hermann D, Kossler T, Young C, Hochberg ME (2004) Theor Appl Genet 109:806
105. Daniels R, Boffey C, Mogg R, Bond J, Clarke R (2005) The potential for dispersal of herbicide tolerance genes from genetically modified, herbicide tolerant oilseed rape to wild relative. Centre for Ecology and Hydrology (CEH) Dorset, Dorchester UK, p 23
106. Halfhill MD, Millwood RJ, Raymer PL, Stewart CN (2002) Environ Biosafe Res 1:19
107. Halfhill MD, Zhu B, Warwick SI, Raymer PL, Millwood RJ, Weissinger AK, Stewart CN (2004) Environ Biosafe Res 3:73
108. Hansen LB, Siegismund HR, Jørgensen RB (2001) Genet Resour Crop Evolut 48:621
109. Hansen LB, Siegismund HR, Jørgensen RB (2003) Heredity 91:276
110. Warwick SI, Simard MJ, Legere A, Beckie HJ, Braun L, Zhu B, Mason P, Seguin-Swartz G, Stewart CN (2003) Theor Appl Genet 107:528
111. Moyes CL, Lilley JM, Casais CA, Cole SG, Haeger PD, Dale PJ (2002) Molec Ecol 11:103
112. Darmency H, Lefol E, Fleury A (1998) Molec Ecol 7:1467
113. Gueritaine G, Bazot S, Darmency H (2003) New Phytologist 158:561
114. Gueritaine G, Sester M, Eber F, Chevre AM, Darmency H (2002) Molec Ecol 11:1419
115. Reichman JR, Watrud LS, Lee EH, Burdick CA, Bollman MA, Storm MJ, King GA, Mallory-Smith C (2006) Molec Ecol 15:4243
116. Rufener A, Mazyad P, Ammann K (1999) In: Ammann K, Jacot Y, Simonsen V, Kjellson G (eds) Methods for risk assessment of transgenic plants III. Ecological risks and prospects of transgenic plants, vol 3. Birkhäuser Verlag, Basel, p 95
117. Elton CS (1958) The ecology of invasions by animals and plants. Methuen and Co Ltd., London
118. Levine JM, Vila M, D'Antonio CM, Dukes JS, Grigulis K, Lavorel S (2003) Proc Royal Soc Lond Ser B 270:775

119. D'Antonio C, Meyerson LA (2002) Restor Ecol 10:703
120. Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) Ann Rev Ecol Systemat 32:305
121. Levin SA (1988) Trend Biotechnol 6:S47
122. Beckie HJ, Seguin-Swartz G, Nair H, Warwick SI, Johnson E (2004) Weed Sci 52:152
123. Hall L, Topinka K, Huffman J, Davis L, Good A (2000) Weed Sci 48:688
124. Beckie HJ, Warwick SI, Nair H, Seguin-Swartz GS (2003) Ecol Appl 13:1276
125. Legere A (2005) Pest Manage Sci 61:292
126. Hall LM, Habibur Rahman M, Gulden RH, Thomas AG (2005) In: Gressel J (ed) Crop ferality and volunteerism. CRC Press, Boca Raton, FL, p 59
127. Crawley MJ, Hails RS, Rees M, Kohn D, Buxton J (1993) Nature 363:620
128. Crawley MJ, Brown SL, Hails RS, Kohn DD, Rees M (2001) Nature 409:682
129. Warwick S, Stewart CN (2005) In: Gressel J (ed) Crop ferality and volunteerism. CRC Press, Boca Raton, FL, p 9
130. Heard MS, Rothery P, Perry JN, Firbank LG (2005) Weed Res 45:331
131. Watkinson AR, Freckleton RP, Robinson RA, Sutherland WJ (2000) Science 289:1554
132. Dewar AM, May MJ, Woiwod IP, Haylock LA, Champion GT, Garner BH, Sands RJN, Qi AM, Pidgeon JD (2003) Proc Royal Soc Lond Ser B 270:335
133. Firbank LG, Forcella F (2000) Science 289:1481
134. May MJ, Champion GT, Dewar AM, Qi A, Pidgeon JD (2005) Proc Royal Soc Ser B 272:111
135. Squire GR, Brooks DR, Bohan DA, Champion GT, Daniels RE, Haughton AJ, Hawes C, Heard MS, Hill MO, May MJ, Osborne JL, Perry JN, Roy DB, Woiwod IP, Firbank LG (2003) Philos Trans Royal Soc Lond Ser B 358:1779
136. Hawes C, Haughton AJ, Osborne JL, Roy DB, Clark SJ, Perry JN, Rothery P, Bohan DA, Brooks DR, Champion GT, Dewar AM, Heard MS, Woiwod IP, Daniels RE, Young MW, Parish AM, Scott RJ, Firbank LG, Squire GR (2003) Philos Trans Royal Soc Lond Ser B 358:1899
137. Chassy B, Carter C, McGloughlin M, McHughen A, Parrott W, Preston C, Roush R, Shelton A, Strauss SH (2003) Nat Biotechnol 21:1429
138. Freckleton RP, Sutherland WJ, Watkinson AR (2003) Science 302:994
139. Firbank LG, Heard MS, Woiwod IP, Hawes C, Haughton AJ, Champion GT, Scott RJ, Hill MO, Dewar AM, Squire GR, May MJ, Brooks DR, Bohan DA, Daniels RE, Osborne JL, Roy DB, Black HIJ, Rothery P, Perry JN (2003) J Appl Ecol 40:2
140. Carpenter J, Felsot A, Goode T, Hammig M, Onstad D, Sankula S (2002) Comparative environmental impacts of biotechnology-derived and traditional soybean, corn, and cotton crops. Council for Agricultural Science and Technology, Ames, Iowa, p 200
141. Phipps RH, Park JR (2002) J Animal Feed Sci 11:1
142. Holland JM (2004) Agric Ecosyst Environ 103:1
143. Heap I (2006) The International Survey of Herbicide Resistant Weeds. www.weedscience.com
144. Owen MDK, Zelaya IA (2005) Pest Manage Sci 61:301
145. Bradshaw LD, Padgette SR, Kimball SL, Wells BH (1997) Weed Technol 11:189
146. CFIA (2003) Technical workshop on the management of herbicide tolerant crops. Canadian Food Inspection Agency, Ottawa, p 28
147. VanGessel MJ (2001) Weed Sci 49:703
148. EPA (2006) Office of Pesticide Programs. www.epa.gov/pesticides/
149. Health Canada (2006) Pest Management Regulatory Agency. www.pmra-arla.gc.ca/english/index-e.html

150. HRAC (2006) Guideline to the management of herbicide resistance. Herbicide Resistance Action Committee, Brussels, Belgium, www.plantprotection.org/hrac/
151. Benbrook C (2001) Pesticide Outlook 12:204
152. Benbrook C (2003) BioTech InfoNet, p 42
153. Brimner TA, Gallivan GJ, Stephenson GR (2005) Pest Manage Sci 61:47
154. Brookes G, Barfoot P (2005) AgBioForum 8:187
155. Fernandez-Cornejo J, Mc Bride WD (2002) Economic Research Service. United States Department of Agriculture, Washington, DC, p 61
156. Duke SO (2005) Pest Manage Sci 61:211
157. Qaim M, Traxler G (2005) Agr Econ 32:73
158. Trigo EJ, Cap EJ (2003) AgBioForum 6:87
159. FAO (2004) The state of food and agriculture – agricultural biotechnology, meeting the needs of the poor? Food and Agric Organization of the United Nations, Rome, p 208
160. Raney T (2006) Curr Opin Biotechnol 17:174
161. Fitt GP, Wakelyn PJ, Stewart JM, James C, Roupakias D, Hake K, Zafar Y, Pages J, Giband M (2004) Global status and impacts of biotech cotton: report of the second expert panel on biotechnology of cotton. International Cotton Advisory Committee, Washington, DC, p 65
162. Wu KM, Guo YY (2005) Ann Rev Entomol 50:31
163. Pray CE, Huang JK, Hu RF, Rozelle S (2002) Plant J 31:423
164. Hossain F, Pray CE, Lu Y, Huang J, Fan C, Hu R (2004) Int J Occup Environ Health 10:296
165. Huang JK, Hu RF, Rozelle S, Pray C (2005) Science 308:688
166. Bennett R, Morse S, Ismael Y (2003) 7th ICABR International Conference, Ravello, Italy, p 11
167. Burnside OC (1996) In: Duke SO (ed) Herbicide-resistant crops. CRC Lewis Publishers, Boca Raton, FL, p 391
168. CCOC (2001) Canola Council of Canada, Winnipeg, p 55
169. Fawcett R, Towery D (2002) Conservation Technology Information Center, West Lafayette, IN, p 20
170. Fernandez-Cornejo J, Caswell M (2006) United States Department of Agriculture–Economic Research Service, Washington, DC, p 30
171. Gianessi LP (2005) Pest Manage Sci 61:241
172. Goklany IM (2002) Nat Biotechnol 20:1075
173. Donegan KK, Palm CJ, Fieland VJ, Porteous LA, Ganio LM, Schaller DL, Bucao LQ, Seidler RJ (1995) Appl Soil Ecol 2:111
174. Palm CJ, Schaller DL, Donegan KK, Seidler RJ (1996) Can J Microbiol 42:1258
175. Sims SR, Holden LR (1996) Environ Entomol 25:659
176. Mikkelsen TR, Andersen B, Jørgensen RB (1996) Nature 380:31
177. Hauser TP, Shaw RG, Østergard H (1998) Heredity 81:429
178. Hauser TP, Jørgensen RB, Østergard H (1998) Heredity 81:436
179. Snow AA, Andersen B, Jørgensen RB (1999) Mol Ecol 8:605
180. Pertl M, Hauser TP, Damgaard C, Jørgensen RB (2002) Heredity 89:212
181. Hauser TP, Damgaard C, Jørgensen RB (2003) Am J Botany 90:571
182. Halfhill MD, Sutherland JP, Moon HS, Poppy GM, Warwick SI, Weissinger AK, Rufty TW, Raymer PL, Stewart CN (2005) Mol Ecol 14:3177
183. Ammitzbøll H, Mikkelsen TN, Jørgensen RB (2005) Environ Biosafe Res 4:3
184. Rieger MA, Potter TD, Preston C, Powles SB (2001) Theor Appl Genet 103:555

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