

Chapter 10

Biodiversity, ecosystem services and genetically modified organisms

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Introduction

Genetically modified (GM) crops have been commercially grown for 10 years. During this time the debate about them and about genetic engineering in general has continued to rage. The general public eagerly follows the developments as well as the arguments; the level of attention is possibly unparalleled since the appearance of the atomic bomb. Some argue that this is the triumph of ignorance, the result of manipulation by environmental protection organizations such as Greenpeace and/or media hype. Sometimes ‘risk assessment’ is pictured as a strategy to block the spread of growing GM crops. Few ecologists subscribe to any of the aforementioned. The debate about the benefits, risks and overall impact of genetic engineering is complex and so it should be. After all, genetic engineering introduces new combinations of genes that may irreversibly be a part of future evolution, and affect the environment and natural resources. The scale of this issue is thus huge and beyond the short-term scientific and political agendas: it triggers ideological, ethical and religious evaluations. In this chapter, we consider one limited but significant part of this problem circle – the potential environmental impact – and link it to the concepts of biodiversity and ecosystem services.

The overall reason to test GM plants before field release is because humankind’s total impact on ecosystem services from previous introductions of new technologies is substantial (Millennium Ecosystem Assessment (MEA) 2005), including habitat destruction, introduction of exotic species, chemical pollution, and global warming, all of which, in themselves and in combination, lead to loss of biodiversity, but also to substantial pressure on all kinds of ecosystems and their services. We have learned from over 100 years of industrial-technological development that all environmentally relevant technologies come with a price – many of which outweigh the benefits in the long run (Harremoës 2002). Consequently, all new potential environmental stressors need to be carefully assessed.

Ecosystem services are ecological processes that operate on vast scales, and we derive substantial benefits from them. Production of goods such as fish and timber, generation of soils and maintenance of their fertility, decomposition, detoxification of wastes, mitigation of climatic extremes, biological control of potential pests, weeds and pathogens, and crop pollination are just some examples of ecosystem services. Their continued functioning is essential for humankind’s survival – they cannot be replaced by technology. Until recently, ecosystem services have been treated as inexhaustible, but the global human population size and its use of resources have reached the point where ecosystem services show evident signs of strain.

Agriculture is one of the human activities that have a large ‘ecological footprint’ (Wackernagel & Rees 1997), meaning that it is a crucial factor in the global ecology. Agriculture is an important driver of environmental quality. In developed countries, there are few farmers (typically < 5% of the population) and they produce food and feed in mostly large-scale, high-input agricultural

systems, including expensive machinery and combustion of fossil fuels. In the developing countries, the situation is different. For example, approximately 70% of Africa's population is engaged in agriculture. Natural processes that underpin agricultural diversity and productivity are both recognized and needed in these regions as most of them have no means to compensate with external inputs.

The concept of biodiversity

According to a recent definition, biological diversity as a concept refers to the variety and variability of living organisms (MEA 2005). Diversity is a multifaceted concept, and ranges from intra-cellular (genetic diversity) to supra-individual (community, landscape and ecosystem diversities) levels (Magurran 2003). Ecologists have long struggled with the concept of diversity and how to quantify it. After decades of intensive search for the best index or formula describing diversity, it was finally realized that there is no single, 'best' diversity description. There exists a 'diversity of diversities' (Juhász-Nagy 1993), including genetic, physiological, species, functional group, landscape, and ecosystem diversity (Box 10.1). In the interests of preserving biodiversity, we also have to recognize the significance of the processes that create, maintain and further develop biodiversity. In a short-term perspective, this means the ecological processes (i.e. competition, predation, etc.); over the long-term, it includes the process of evolution (Bøhn & Amundsen 2004). Too often, biodiversity is viewed as a static characteristic of communities. However, biodiversity is the emergent outcome of dynamics at ecological and evolutionary timescales.

Box 10.1 Definitions

Genetic diversity: This concept refers to the variability of genes within a species. The total number of genes that can be found in one species is never present in one individual: individuals of the same species contain a lot of identical genes but also many different ones. Genetic variability is the key to the adaptation potential to changing conditions. A species that has lost its genetic diversity is either unable or severely impaired to adapt to new conditions.

Physiological diversity: As genes only provide a 'set of instructions', the realization of this programme, depending on the environmental conditions during development, always results in slightly different physiological outcomes in individuals. They will differ in their physiology: heat tolerance, ability to resist starvation, digestion efficiency, etc.

Species-individual diversity: Communities of living organisms are composed of individuals that are classified into species. Intuitively, the more species there are in a community, the more diverse it is. The minimum diversity in a community occurs when all individuals belong to the same species. A theoretical maximum level of species diversity would be reached when all individuals belong to different species. A characterization of species diversity depends on our ability to recognize individuals as belonging to different species, and to count them.

Functional diversity: Species have different characteristics and are distinguishable, but they may be grouped according to their activity in habitats and food webs. One possibility is to group them by their feeding habits. Plants use inorganic materials and energy (mostly sunlight) to grow, in the process of producing more plant material. They can be classified into the functional group of *primary producers*. Organisms feeding on plants form the *primary consumers*, while those feeding on these are called *secondary consumers*. At the top of some food-chains are the *top predators*, often large animals. Functional groups can be further refined. One aspect of functional diversity is the diversity of such groups themselves (not all of them are present everywhere), while another is to assess the diversity within each group.

Landscape diversity: At a wider spatial scale, different habitats (for example, forests, meadows, streams, marshes, cultivated fields) form landscapes. Both the types and distribution of these compositional elements are important in determining the diversity at this level. For example, if the elements occur in one block each, the landscape-level diversity is considered lower than when the same total area of the composing elements occurs in several smaller blocks. The transition between landscape and ecosystem diversity is not always straightforward.

Ecosystem diversity: Ecosystems can be larger units, composed of several landscapes (but some argue the opposite). An ecosystem is defined as a recognizable, self-sustaining unit, but it is more plausible to consider this a theoretical.

Different biodiversity concepts, as detailed in Box 10.1, range from intra-individual (genetic) to supra-individual (species, landscape, etc.) levels, and all are relevant, depending on *context*. However, it has to be added that the most frequent use of the word biodiversity (sometimes even without definition) implies the species-individual based diversity, i.e. the word ‘diversity’ means the number of species. In nature, most communities contain a small number of ‘common’ and a much larger number of ‘rare’ species. Some diversity indices account for such differences but all diversity representations contain different simplifications. For example, for most diversity indices, the species identity is not important – only the density of the species present is taken into account. Two communities with the same number of species and identical relative densities would have the same diversity value even if there were no common species in them.

The functions of biodiversity

Diversity, in all of its manifestations, is valued for several different reasons. Biodiversity is also important for the functioning of ecological systems (Loreau et al. 2002), but the central question is: just how important? There are different theories to explain the significance of biodiversity for ecological systems. These theories are vigorously studied, hotly debated and not always mutually exclusive (Loreau et al. 2002; Hooper et al. 2005). The main ideas are briefly presented as follows.

1. Biodiversity has a (positive) impact on productivity

Several experiments have indicated that a more diverse ecological community of plants will produce a higher biomass than a less species-rich one (Loreau et al. 2002). The existing evidence supporting this claim is equivocal and has been debated (Hooper et al. 2005). More species can utilize the available resources more efficiently, but there seem to be some key species that have disproportionate influence on this and consequently also on productivity (Wardle & van der Putten 2002). In a more species-rich assemblage, it is more probable that such species can be found. Another hypothesis claims that a more diverse system will experience less year-to-year fluctuations in plant biomass production than a species-poor one.

2. Insurance against change (resistance and resilience)

In terms of energy efficiency, most biodiversity is unnecessary (redundant) for ecological functioning *under stable conditions*. However, elements that seem redundant under one set of conditions may become necessary if conditions change, since the organisms have to adapt. Changing conditions occur naturally, for example by extreme weather conditions, but also due to human activities, such as global warming and introduction of exotic species. It may be hard to separate natural- and human-triggered changes. For example, global warming tends to increase the occurrence of extreme weather events. Whereas resistance refers to the ability to resist change under the pressure of stressful conditions, resilience refers to the ability to return to a previous

state after a disturbance. Both traits are important for continued functioning of ecological systems.

3. Providing ecosystem services

Ecosystem services are linked to points 1 and 2 above. A more detailed explanation of their nature and importance will follow.

Human domination of the Earth

We now recognize that human impact over all of the Earth is substantial, whether we consider land conversion, use of resources, or impact on other species. Today, 25% of the global terrestrial surface has been converted to cropland (Fig. 10.1). The conversion rate is accelerating: more land was converted in the 30 years since 1950 than during the 150 years from 1700 to 1850. More than two-thirds of the area of two biomes (temperate forest; tropical dry forest) and more than half of the area of four others (Mediterranean forests; flooded grassland and savannas; tropical and subtropical savannas and grasslands; tropical and sub-tropical coniferous forests) had been converted by 1990. Our impact on other parts of the globe is also large. For example, 20% of all coral reefs had been exterminated, a further 20% damaged, and 35% of the global mangrove area had been destroyed by 1990 (MEA 2005).

Increases in fertilizer application have followed suit, and biologically available nitrogen in terrestrial systems has doubled, and that of phosphorus tripled since 1960. However, this change is extremely disproportionately distributed, with overuse in industrial countries to the point of polluting water bodies and lack of it in developing countries to the point where agriculture production is severely limited (e.g. Africa). For example, the average application in 1992 of N fertilizer was 323 kg/ha in Western Europe while only 7 kg/ha in Africa (FAO 1993). Nevertheless, at a global level, more than 50% of all the synthetic nitrogen fertilizer ever used has been used since 1985, and 60% of the increase in the atmospheric concentration of CO₂ since 1750 has taken place since 1959 (MEA 2005).

Another limited vital resource is water and we claim more and more of the available freshwater resources. The amount of water in reservoirs has quadrupled since 1960, and today there is 3–6 times more water in reservoirs than in all natural rivers combined (MEA 2005). Water withdrawal from rivers and lakes has doubled since 1960. As a result of combined erosion and river regulation, the sediment load of many major rivers has been substantially altered from pre-human conditions (Syvitski et al. 2005). In some rivers, sedimentation has increased by up to 200% and even large rivers hardly reach the coast. For example, only 10% of the Nile manages to meet the ocean. Increased sedimentation rates have caused death zones in deltas where depositing sediments are often loaded with poisonous chemicals (Syvitski et al. 2005).

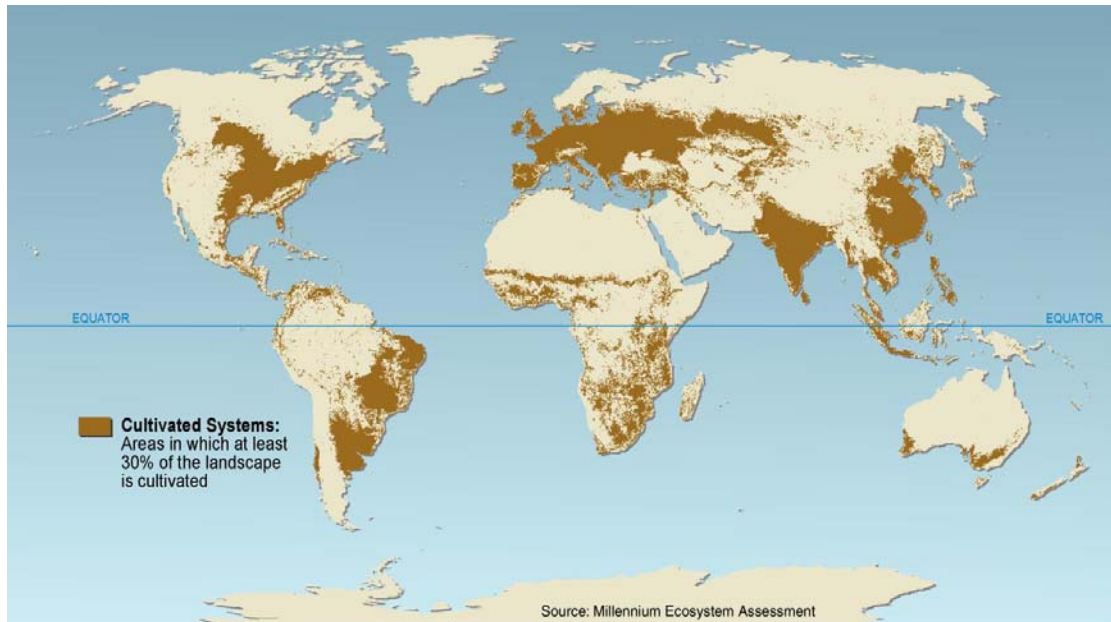


Figure 10.1. The terrestrial areas converted to cropland worldwide. Source: Millennium Ecosystem Assessment, 2005.

Concerns about biodiversity

The impacts of agriculture on resources together with other human activities have had significant impacts on global biodiversity. Introduced species have had particularly broad impact. In historic times, numerous intentional introductions of species deemed useful or merely desirable at new locations have been made. Their effects are often considered beneficial, but we have numerous examples of unwanted, significant negative effects (Baskin 2002), and the number of invasive species is steadily increasing (for an example, see Fig. 10.2). Together with unintended introductions, invasions have become a significant problem, and an element of global change (Vitousek et al. 1997). One significant consequence of this is the increasing homogenization of the distribution of species on Earth (Lövei 1997). The breakdown of biogeographical barriers leads to reduced global biodiversity (Vitousek et al. 1997).

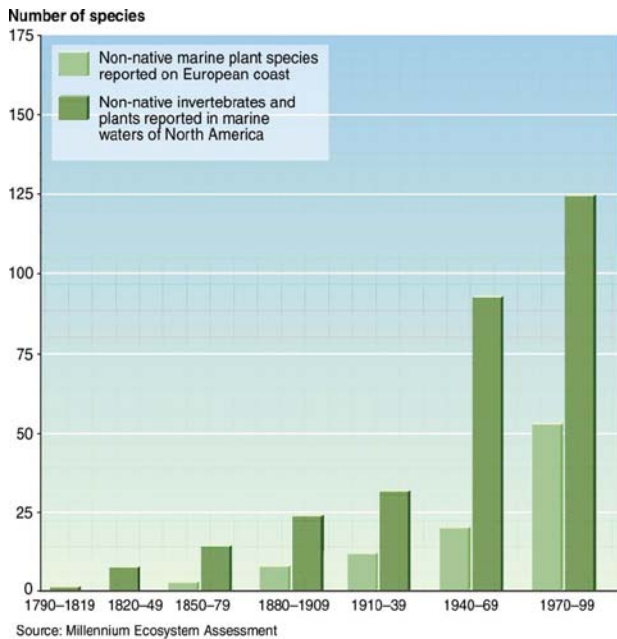


Figure 10.2. The number of non-native species reported from marine habitats in Europe and North America, 1790–1999. Source: Millennium Ecosystem Assessment, 2005.

Further signs of stress in the global biodiversity is that the population size or range (or both) of the majority of species across a range of taxonomic groups is declining (MEA 2005). Currently, estimated species extinction rates are 1000 times higher than background rates typical of the planet’s history (Fig. 10.3) (MEA 2005; Lövei 2007). A total of 10–30% of mammal, bird, and amphibian species are currently threatened with extinction (Secretariat CBD 2006).

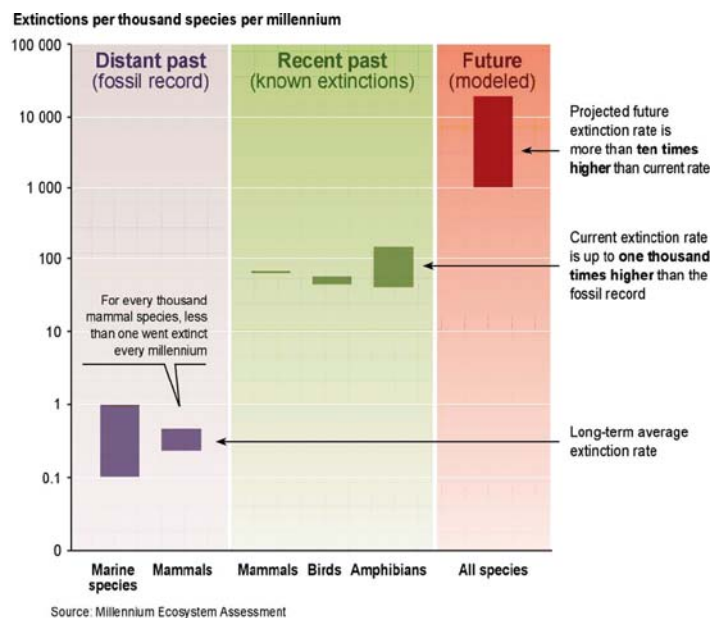


Figure 10.3. Estimated extinction rates: historical, recent and predicted. Source: Millennium Ecosystem Assessment, 2005.

Ecosystem services

Ecosystem services denote ecological processes that humankind benefits from (Daily 1997). These processes operate on vast scales, are irreplaceable, and have been formerly perceived as inexhaustible. Several types of ecosystem services ensure agricultural productivity, including soil formation, decomposition of plant residues, pollination, and natural pest control, to name a few. Several of these are already under pressure and their ability to continue at desired rates is in peril (MEA 2005).

The Millennium Ecosystem Assessment (MEA) recognizes four categories of ecosystem services (Box 10.2).

Box 10.2

Provisioning services are simply used or harvested, and in most cases humans do not do anything to manage them. Provisioning services include the provision (harvesting from the wild) of food, freshwater, medicine, fibre, and timber, energy, or industrial products (e.g. rubber). Genetic resources used for plant breeding also belong to this category.

Supporting services include services that, by their functioning, support the normal functioning of ecosystems. This includes the removal of waste products through detoxification, decomposition, air and water purification, but also soil formation and fertility maintenance, and supporting plant production through seed dispersal, and pollination.

Regulating services provide coastal and river channel stability, moderation of weather extremes, floods and drought, as well as the natural control of pests. Most organisms can occur at high densities but they do not (i.e. they do not become pests). This is due to the activity of natural enemies.

Cultural services provide numerous valuables to humans and human culture. Humankind is psychologically closely linked to nature (the ‘biophilia’ hypothesis, Wilson 1984). Nature is a constant source of aesthetic beauty, provides cultural and spiritual inspiration, inspires scientific discovery, and endless varieties of recreation.

Why do ecosystem services have to be considered in GM impact assessment?

As described, ecosystem services are essential for agricultural production. As the MEA concluded, humankind already is using many of the ecosystem services in a non-sustainable manner. Any further damage must be avoided. Also, the negative trends in biodiversity and natural resources must be taken very seriously. Consequently, when introducing new technologies today, such as GM crops, their potential impact on ecosystem services must be tested (Lövei 2001). Such testing is even more important in tropical countries, where agricultural producers often depend on ecosystem services more closely than farmers in the developed countries. Modern high-input agricultural practices use several external inputs that at least partially replace ecosystem services (fertilizers, pesticides, irrigation, and even pollination). Irrespective of the questionable sustainability of this practice (Tilman et al. 2002), these external inputs are often not available to farmers in developing countries, hence they have to rely more on natural ecosystem services. As GM crops will be grown outdoors, in contact with surrounding ecosystems, and they certainly have the potential to substantially modify current agricultural practices (Hawes et al. 2003), the environmental impact of genetic engineering on ecosystem services will have to be examined thoroughly (Hails 2002). Box 10.3 lists the most important potential adverse impacts currently discussed and partly investigated.

Box 10.3 Possible environmental impacts of GM crops

At intra-individual (genetic) level:

- damage to genetic resources (particular genes, gene combinations, seeds, varieties, etc.)
- uncontrolled gene flow to other species

At population level:

- species shifts due to altered traits, consciously or accidentally (via unintended gene flow)
- development of secondary pests
- development of resistant populations, curtailing the usefulness of the GM trait
- damaging of protected/endangered species (nature conservation)

At ecosystem level:

- decline in agricultural biodiversity due to the homogenization of the primary producer base (a centralized production of a relatively few, patented events, traits and varieties).

Loss of ecosystem services:

- damaging naturally-occurring biocontrol organisms
- loss of pollination services
- impact on soil organisms involved in recycling of soil nutrients and maintaining soil fertility (can be positive, due to reduced soil tillage, or negative)

For agricultural production systems:

- decrease in pesticide use, soil tillage, environmental contamination
- threatening of GM-free production reducing future choices
- loss or reduction in practices that uphold and develop varieties (i.e. diversity) with adaptations to local environmental conditions
- food or agricultural production in areas where it was not possible earlier (e.g. due to high levels of stress, lack of water, etc.)
- rearrangement of agricultural production systems, in space and time, and its resulting consequences for landscape management

Incorporating ecosystem services into risk/impact assessment poses several challenges:

The structure and function in relevant ecosystems and food-webs have to be recognized. For example, an ecosystem may contain predator-prey relationships that keep a number of pests under control (i.e. at low densities, so we do not recognize them as a pest). Productivity may also depend on insect pollination services (e.g. cotton).

The significant functional links must be established where structure and function are reasonably well understood. Following the aforementioned example, it may turn out that pollination is much more significant than pest control for productivity in the ecosystem where a GM crop is to be introduced.

Most important species fulfilling identified relevant ecological roles that should be subjected to pre-release testing have to be identified. However, we should not forget that even the most important functions will typically be performed by numerous species. Again, following the

mentioned example, pollination services may be provided by more than 30 bee species, but the most important could be just one, or a handful of them.

Pre-release testing should focus on these functionally important species. When such species are identified, suitable testing and monitoring methods must be developed for them. If there is no option to identify species responsible for the execution of important ecological services – as, for instance, is the case with most soil microorganisms – the relevant processes must be identified and a potential adverse impact of the GMO tested. There may or may not be suitable laboratory culture systems or field monitoring methods already available for these functionally important species or processes. If such tools are lacking, they should be developed.

Current testing regimes for GM plants

Understanding the importance of ecosystem services and the need to avoid any further adverse impacts on them through the introduction of GMOs begs the question as to what degree current regulatory testing actually addresses the issues raised so far in this chapter and how they are tested. Today, applicants applying for regulatory approval of GM plants follow largely the guidelines originally developed for testing the environmental effects of chemicals (pesticide model). The strategy used in ecotoxicology testing of chemicals is to expose single species (standard set) to single chemicals in a hierarchical tiered system. Tests commence with simple inexpensive range finding tests on single species and measure acute toxicological response to a chemical stressor. Further testing proceeds to more expensive higher tiered levels (including some chronic toxicity tests), only if first-tier experiments yield results of concern. In practice, this results in the testing of a standard set of species exposed individually to high concentrations of the toxin.

In the case of a GM plant producing the *Bacillus thuringiensis* toxin (Bt plant), for example microbially produced Bt-toxins are fed directly to testing organisms (bi-trophic exposition) in an experimental set-up originally developed to assess acute toxicity of synthetic chemicals. Acute toxicity measures the physiological toxicological response of an organism after being directly exposed to the isolated test substance within a short period of time (sometimes hours rather than days).

The standard set of species is representative of model ecosystem compartments, such as a generalized aquatic or terrestrial compartment. An algae species is tested as a representative for primary producers in aquatic systems (plants), water fleas (*Daphnia* spp.) as a representative of a primary consumer, and a fish species representing a secondary consumer (i.e. predator). The endpoint measured is mortality after hours or a few days (Table 10.1) (Andow & Hilbeck 2004). Further criteria for their selection as standard organisms are their documented sensitivity to certain groups of chemicals and/or their capability of accumulating high concentrations of heavy metals (e.g. springtails or earthworms). Hence, the concept of toxicity (and ecotoxicity) testing of chemicals is exceeding the notion of a case-specific testing regime related to the given receiving environment. A standard test performed in temperate Europe is (erroneously) considered applicable to tropical Africa, and vice versa.

Table 10.1. Some standardized guidelines for ecotoxicological testing of pesticides and GMOs (OECD 1998).

<i>Test organism</i>	<i>Test method</i>	<i>Duration</i>	<i>OECD Guideline No.</i>
<i>Water fleas, Daphnia</i>	<i>Acute immobilization/toxicity</i>	<i>24-96 h</i>	<i>202</i>

<i>Fish sp. (rainbow trout)</i>	<i>Acute toxicity</i>	<i>24-96 h</i>	<i>203</i>
<i>Fish sp.</i>	<i>Toxicity to juvenile life stages</i>	<i>4-12 wk</i>	<i>210</i>
<i>Eisenia foetida (compost worm)</i>	<i>Acute toxicity</i>	<i>7-14 d</i>	<i>207</i>
<i>Bobwhite quail & mallard duck</i>	<i>Acute toxicity</i>	<i>14-21 d</i>	<i>205</i>
<i>Honey bees</i>	<i>Acute toxicity (oral & contact)</i>	<i>4-24 h</i>	<i>New (1998)</i> <i>213</i> <i>214</i>

<http://ecb.jr.it/testing-methods>
www.oecd.org/dataoecd/9/11/33663321.pdf

The pesticide model as a testing guideline for insecticidal GM plants is problematic for a number of reasons. Plants are not chemicals and regulations and scientifically sound testing procedures must account for the differences:

- i) In GM plants, the plant-expressed transgene product is an integral component of the plant and coupled to its metabolism. This leads to variable expression levels of the transgene product that is additionally modulated by environmental conditions, including seasonal changes in temperature, soil type, moisture, and light. On the other hand, due to the wide use of universally functioning viral promoters and terminators, the transgene products of most, if not all, currently commercially available GM plants are expressed essentially in all plant parts throughout the entire growing season. When comparing with pesticides, this is equivalent to a long persistence of the pesticidal substance and an almost complete coverage of the plant.
- ii) GM plants are capable of self-reproduction. This is a fundamental difference to chemicals. Because of this capability, biological traits and organisms can increase in the environment and potentially spread and exist for unlimited time. In contrast, chemicals cannot reproduce and, thus, their absolute amount will, at best (or worst), remain stable for a long time, but over time will always decline. Most disappear within humanly conceivable time periods due to degradation.
- iii) GMOs can actively spread and with them their transgene products will also spread. In addition, all passive mechanisms of spread for chemicals also apply to transgene products released into the environment from the living GM plants (e.g. exudates, leaching from living and dead material). The potential of human-aided spread of seeds, plants and animals (as already realized and exemplified in invasion biology) should not be underestimated (Baskin 2002, see Box 10.4).

Box 10.4 Spread of GM plants: Control or chaos?

Unwanted and uncontrollable spread of GM plants is a highly visible process on a global scale. By the end of 2006, over 100 cases of confirmed, unwanted contamination and 26 cases of illegal releases were registered (mostly by civil society organizations) (see GM contamination register, <http://www.gmcontaminationregister.org/>). A total of 39 countries on five continents have been affected, almost twice the number of countries that currently grow GM crops. In 2005, there were 7 documented cases of contamination and 8 illegal releases. In 2006, the number of

contamination cases more than doubled to 15. Most prominently, two unapproved GM events were found in rice (a herbicide-tolerant transgene from the USA and a Bt transgene from China) – these were detected at the consumer level (in shipments intended for human consumption). These were possible to detect because the necessary detection methods were available. More problematic is the detection of plants with GM traits that have not yet been commercialized. Several such lines are at the field trial stage, among them many pharmaceutical traits, for which the necessary detection methods are not yet widely available and therefore detection is more difficult. The global, illegal or unwanted spread of transgenes and their products shows a worrying tendency and it is likely that this trend will continue, perhaps even accelerate, over the coming years.

For these reasons, it is extraordinarily more difficult if not impossible to determine the exact exposure concentrations in a given environmental compartment for GM plants as compared to chemicals. In contrast, chemical pesticides (i.e. sprayed in the field) are controlled by the applicator: the timing, the point location, etc. Degradation begins immediately after application and the mode of action is typically acute (also for non-target species). A scientifically sound testing strategy and methodology for GM plants require case-specific risk assessment and must account for the whole transgenic organism. It must also treat a GM plant within an integrated biological system consisting of the plant, the novel trait and the receiving environment. Sub-lethal, chronic effects might be even more important to test for than acute effects, as the mode of action for the toxin is not immediate (it normally takes two days or longer before the ‘target’ dies).

Selection of test organisms

Even for chemical testing, it is problematic to use test organisms of higher trophic levels because the test substance is often not ingested directly by these organisms but is ingested via one or several intoxicated prey species. These prey species may contain the test substance, or metabolites thereof, in unknown concentrations. From our knowledge of persistent chemicals such as DDT and PCB, we know that they can accumulate and even become more toxic along the food chain. This means they can reach concentrations and toxicity levels that, at the end of the food chain, are multi-fold above the levels originally introduced into the ecosystem (Woodwell et al. 1967). We also know from research on insect-plant interactions, that insects can use toxic proteins in their host plants to turn them into defence mechanisms against their enemies. One example is the monarch butterfly (*Danais plexippus*), whose larvae accumulate an alkaloid from the host plant, milkweed, that makes them unpalatable. We do not know how herbivore species, which are not affected by novel transgene compounds, may be using them against their enemies. These complications make it currently unlikely that a few selected species could universally be used for pre-release risk assessment of GM plants.

Representativeness of test materials

As already mentioned, in toxicological and ecotoxicological testing of pesticidal GM plants, high concentrations of the microbially produced transgene product, e.g. the Bt-toxin, are applied. The significance of such tests is limited because the Bt-toxin expressed in GM plants can be quite different from the microbially derived toxin. For example, the Bt-toxin of the Cry1-class used in the regulatory tests has been derived either from the original *Bacillus* or from genetically modified *Escherichia coli*. After the microbial synthesis, the product is a protoxin of 130 kDa in size which is inactive (Höfte & Whiteley 1989; Müller-Cohn et al. 1996). Before use in the tests, the protoxin is cleaved by trypsin to create the toxic fragment of 65 kDa size. However, in transgenic Bt-plants, fragments of different sizes of the Cry1-class toxins are produced. For example, the Bt-corn event MON810 expresses a 91 kDa fragment, whereas Bt-corn event 176

expresses a 64 kDa fragment (Andow & Hilbeck 2004). From other events, it is known that the Bt-toxins degrade within the plant to fragments of even smaller size (36, 40, 55, 60 kDa) of unknown activity¹(Andow & Hilbeck 2004; AGBIOS 2006). In conclusion, this means that the Bt-toxins expressed in GM plants may vary significantly in size and activity from the test substances used to assess safety, i.e. in standard toxicological and ecotoxicological testing. In summary, a GM plant is not a chemical. Any environmental testing must therefore account for the difference. Test strategies for case-specific risk assessment of GM plants must include the transgene product, the transformed plant and the environment of deployment as an integrated system. This is even more important in the case of GM plants that do not express a toxin, but have, for instance, an altered metabolism (e.g. herbicide tolerant plants or altered starch composition). In these cases, the adoption of test principles from chemical testing is even less relevant because environmental effects of these GM plants may become evident on other levels altogether. Following the logic for strict toxicity testing, for those GM plants that do not express a novel toxin, no testing would be required at all. This is the case for most herbicide tolerant plants to date. As the ecological impact will arise through the application of registered chemicals, no toxicity or ecotoxicity testing will need to be conducted with these plants.

A proposed new approach for environmental impact testing

Conceptual and methodological uncertainties of studying the ecological effects of GM crop plants on non-target arthropods (insects) have raised several intriguing general problems. What species or ecosystem functions should be chosen to test? By what routes might these species or functions be exposed directly or indirectly to GM crop plant products? How can meaningful scientific hypotheses be constructed to provide rapid assessments of the magnitude of the potential risks? In contrast to toxicological and ecotoxicological methods for addressing these problems, assessment of the impacts of GM crop plants must be case specific and contextualized to the environment in which they will be used. An international project in which two of the authors (Gábor Lövei and Angelika Hilbeck) have been involved, developed an 'ecosystem representative approach' for selecting species and ecosystem function as foci for further testing (Birch et al. 2004; Andow et al. 2006). This approach combines ideas and methods from a 'community approach', which emphasizes analysis of intact biodiversity, a 'functional approach', which emphasizes community reactions, a 'key species approach', which emphasizes the individuality of species, and an 'indicator species approach', which is central in ecotoxicological testing. We used classic qualitative methods of risk assessment formalized in selection matrices and directed questions, which provide transparent summaries of scientific data and expert judgement that then serve as basis for constructing testing hypotheses and designing proper experiments that address the hypotheses.

The process of ranking and species selection in the above-ground functional groups (herbivores, decomposers, natural enemies, and pollinators), allows the identification and prioritization of non-target species for some key ecological groups; it also reflects the current state of knowledge and expertise available, and identifies gaps in knowledge and uncertainties. When analysing the available information to assess the relative importance of parasitoids in maize in Kenya, for example, the information gaps could be recognized, as well as the realization that the two main maize growing regions, the lowland and the Western highlands, have to be considered separately (Table 10.2). It is also important to consider the process of exposure as part of the overall species selection. The species selection can identify missing information, for example the varying expression of Bt-toxin in different plant tissues in the Kenyan example, and is also crucial for the above-ground exposure analysis. An example of an analysis of significance and exposure is presented in Table 10.3.

¹www.agbios.com/main.php

Table 10.2. An example of the filled-in selection matrix for parasitoids in maize agroecosystems in Kenya, following the system proposed by Birch et al. (2004).

Sub-guild	Species	Occurrence	Abundance	Presence	Linkage	Rank
Lowland, Kenyan coast						
Egg parasitoid	<i>Trichogramma</i> spp.	Certain	Medium	All season	Strong	1
Larval parasitoid	<i>Cotesia flavipes</i>	Certain	Medium	All season	Strong	1
Larval parasitoid	<i>C. sesamiae</i>	Certain	Low-medium	All season	Strong	2
Larval parasitoid	<i>Goniozus indicus</i>	Not completed				
Egg & larval parasitoid	<i>Chelonus curvimaculatus</i>	Not completed	Short rains?			
Pupal parasitoid	<i>Pediobus furvus</i>	Certain	Low	All season	Strong	2
Pupal parasitoid	<i>Dentichasmias busseolae</i>	Occasional	Low	All season	Strong	3
Highland, Western Kenya						
Egg parasitoid	<i>Trichogramma</i> spp., native	Likely	Medium	All season	Strong	2
Egg parasitoid	<i>Telenomus</i> spp.	Not completed				
Larval parasitoid	<i>Cotesia sesamiae</i>	Certain	Medium	All season	Strong	1
Larval parasitoid	<i>C. flavipes</i>	Occasional	Low	All season	Strong	3
Pupal parasitoid	<i>Dentichasmias busseolae</i>	Occasional	Low	All season	Strong	3
Pupal parasitoid	<i>Pediobus furvus</i>	Certain	Low	All season	Strong	2

Table 10.3. An example of the exposure analysis assessment as suggested by Birch et al. (2004). The example is plant-feeding arthropods in maize agroecosystems in Kenya.

Species	Feeding category	Significance	Assessment of exposure
			<i>Spodoptera</i> spp.
		Acarid spp.	Leaf feeder
		Locusts	Leaf feeder
		<i>Sitophilus zeamays</i>	Grain feeder
		<i>Prostephanus truncatus</i>	Grain feeder
		Plant- and leafhoppers	Phloem feeder
		<i>Carpophilus</i> spp.	Saprovore
		Honey bee (<i>Apis mellifera</i>)	Pollen feeder
		Wild bee spp.	Pollen feeder
		Coccinellid spp.	Pollen feeder, predator
		Forficulidae	Pollen feeder, predator
		<i>Trichogramma</i> spp.	Parasitoid
		<i>Trichogrammatoidea</i> spp	
		<i>Cotesia flavipes</i>	Parasitoid
		<i>Cotesia sesamiae</i>	Parasitoid
		Other predators: ants, anthocorids, chrysopids	Predators

This underlines the role of this approach to identify and assess the significance of knowledge gaps and uncertainty. Rather than only moving on as a ‘decision has to be made’, significant knowledge gaps will not be overlooked and can trigger specific action, either to stop an assessment procedure, or to initiate specific, targeted research.

The ranking and selection matrix for soil ecosystem functions has a slightly modified format, to rank and select ecosystem functions. Here, key interactions are to be identified in a systematic and transparent way; species and food-webs affected by, e.g. Bt maize, might be studied in a more relevant manner than performed until present.

Conclusions

In this chapter, we suggested that the basis of environmental risk/impact assessment should be the concepts of biodiversity and ecosystem services. Biodiversity is under threat by mainly human activities. Apart from a moral obligation to protect biodiversity, there is also a utilitarian reason, as biodiversity is important for the functioning of ecosystem services. Ecosystem services are vital for our continued existence, but recent summaries have indicated that humankind is using many of them in unsustainable ways. Consequently, it is mandatory that the impact of new kinds of activities, such as growing GM plants, be tested for their impacts on ecosystem services. Ecological systems are, however, complex and often imperfectly known. We have suggested a transparent, knowledge-based assessment procedure by which important functions and the species or groups that are most significant for this function are identified. This provides one way to develop specific pre-release testing and monitoring systems to assess the environmental impact of GM plants. This system also allows for the identification and evaluation of the significance of knowledge gaps, thus making the precautionary approach in risk assessment operational.

References

- Andow, D.A. and Hilbeck, A. (2004) Science-based risk assessment for non-target effects of transgenic crops. *Bioscience* 54: 637-649.
- Andow, D.A., Lövei G.L. and Arpaia S. (2006) Bt transgenic crops, natural enemies and implications for environmental risk assessment. *Nature Biotechnology* 24, 749-751.
- Baskin, Y. (2002) A plague of rats and rubber vines. Island Press, Washington, D.C. 330 pp.
- Bøhn, T. and Amundsen, P. A. (2004) Ecological interactions and evolution: Forgotten parts of biodiversity? – *Bioscience* 54: 804-805.
- Birch ANE, R. Wheatley, B. Anyango, S. Arpaia, D. Capalbo, Emanu Getu D., E. Fontes, P. Kalama, E. Lelmen, Lövei G, et al. (2004) Biodiversity and Non-Target Impacts: a Case Study of Bt Maize in Kenya. Ch 5 in: Hilbeck, A., Andow, D.A., (Eds.) *Environmental Risk Assessment of Transgenic Organisms: A Case Study of Bt Maize in Kenya*. CABI International, Wallingford. U.K., pp. 117-185.
- Daily, G.C. (Ed.) (1997) *Nature's services*. Island Press, Washington, D.C.
- FAO (1993) *FAO Fertiliser Yearbook*. Volume 43, FAO Statistics Series no. 119. FAO Rome.
- George, M.W, Wurster, Ch.F. jr., Isaacson, P.A. 1967. DDT Residues in an East Coast Estuary: A Case of Biological Concentration of a Persistent Insecticide. *Science* 156: 821 – 824
- Hails, R.S. (2002) Assessing the risks associated with new agricultural practices. *Nature* 418, 685-688.
- Harremoës, P., Gee, D., MacGarvin, M., Stirling, A., Keys, J., Wynne, B. & Guedes Vaz, S. (2002): Late lessons from early warnings: the precautionary principle 1896-2000. – 22. – Copenhagen, Denmark (European Environment Agency): 211 S.
- Hawes C, Houghton AJ, Osborne JL, et al. (2003) Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of The Royal Society of London Series B-Biological Sciences* 358: 1899-1913.
- Hilbeck, A., Andow, D.A. and Fontes, E.M.G. (eds) (2006) *Environmental Risk Assessment of Genetically Modified Organisms Volume 2: Methodologies for Assessing Bt Cotton in Brazil*. CABI Publishing, Wallingford, UK.
- Höfte, H. and Whiteley, H.R. (1989) Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Reviews*, 53, 242-255.
- Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setälä H, Symstad AJ, Vandermeer J, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs* 75: 3-35.

- Juhasz-Nagy P (1993) Notes on compositional diversity. *Hydrobiologia* 249: 173-182.
- Loreau M, Naeem S, Inchausti P. , (Eds.) (2002) Biodiversity and ecosystem functioning. Oxford Univ. Press.
- Lövei, G.L. (1997) Global change through invasion. *Nature* 388, 627-628.
- Lövei, G. L. (2001) Ecological risks and benefits of transgenic plants. *New Zealand Plant Protection* 54:93-100.
- Lövei, G.L. (2007) Extinctions, modern examples of. In: Levin, SA (Ed.) *Encyclopaedia of biodiversity*, 2nd edition, Elsevier, Oxford, U.K. (in press)
- Magurran AE (2003) *Measuring biological diversity*. Blackwell, Oxford.
- Millennium Ecosystem Assessment (MEA) (2005) *Ecosystems and human well-being: our human planet*. Island Press.
- Müller-Cohn, J., Chaufaux, J., Buisson, Ch., Gilois, N., V. Sanchis, and Lereclus, D (1996) *Spodoptera littoralis* (Lepidoptera: Noctuidae) resistance to CryIC and cross-resistance to other *Bacillus thuringiensis* crystal toxins. *Journal of Economic Entomology* 89, 791-797.
- OECD (2006) OECD guidelines for testing of chemicals
<http://www.oecd.org/dataoecd/9/11/33663321.pdf> (accessed June 2007)
- Secretariat of the Convention on Biological Diversity (CBD) (2006) *Global Biodiversity Outlook 2*. Montreal, 81 pp.
- Syvitski JPM, Vorosmarty CJ, Kettner AJ, et al. (2005) Impact of humans on the flux of terrestrial sediment to the global coastal ocean. *Science* 308: 376-380.
- Tilman, D., Cassman K.G., Matson, P.A., Naylor, R., Polasky, S. (2002) Agricultural sustainability and intensive production practices. *Nature* 418, 671-677.
- Vitousek PM, D'Antonio CM, Loope LL, Rejmanek M, Westbrooks R. (1997) Introduced species: A significant component of human-caused global change *Nz J Ecol* 21,1-16.
- Wackernagel M, Rees W.E. (1997) Perceptual and structural barriers to investing in natural capital: economics from an ecological footprint perspective, *Ecol. Econ.* 20 (1997), pp. 3-24.
- Wardle DA, van der Putten WH (2002) Biodiversity, ecosystem functioning and above-ground-below-ground linkages. Pp 155-168 in: Loreau M, Naeem S, Inchausti P. , Eds., *Biodiversity and ecosystem functioning*. Oxford Univ. Press
- Wilson, Edward O. (1984). *Biophilia*. Cambridge: Harvard University Press