#### Facts Series

### Effect of genetically modified crops on the environment



6.6



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### Summary

This report forms a two-part series on the safety of genetically modified (GM) crops along with the background report on "Food safety of genetically modified crops"<sup>1</sup>, which has already been published. In this report we discuss what impact GM crops have on the environment.

All agriculture, including cultivating a particular crop, has an impact on the environment. Planting calendars determine which weeds and insects are present in the field, agricultural machinery compresses the earth, uses fuel, and emits CO<sub>2</sub>, whilst excessive use of fertilizers and pesticides can leave traces in and on the earth. A crop that produces a lot of pollen and nectar will attract pollinators such as bees, while another crop can proliferate and suppress local vegetation. As a result, switching from growing oats to growing maize, for example, has an impact on the environment. In addition, a particular characteristic of a crop (for instance, insect resistance)—which we will call a "crop trait" from here on in—can also affect its impact on the environment. New crop traits are obtained through plant breeding methods. These methods include the most traditional techniques, such as cross-breeding, genetic modification and the latest methods, which modify plant DNA in a more targeted way (for more information, see the background report "From plant to crop: The past, present and future of plant breeding"<sup>2</sup>). The impact on the environment of a crop, whether GM or non-GM, or of a crop trait, whether obtained through GM technology or not, depends first and foremost on the crop and/or the crop trait itself rather than on the technology used to develop the crop. In terms of GM crops, four major traits are commercially available at present. The aim of some traits, such as virus and insect resistance or drought tolerance, is to reduce the impact of agricultural practices on the environment. Other traits, such as herbicide tolerance, are primarily introduced to make food production more efficient. In other words, not all traits can be said to promote environmentally friendly agriculture. However, the statistics on the environmental impact of GM crops paint a different picture from the abundant negative reporting in the media. Overall, the cultivation of GM crops over the last 18 years has delivered substantial benefits for the environment.<sup>3</sup> Insect-resistant crops have resulted in a 230 million kg decrease in the use of insecticides. Herbicide-tolerant crops have led to reductions in fuel use and CO, emissions of 6.3 billion liters and 16.8 million metric tons respectively, by supporting no-till farming. Overall, GM crops have produced an environmental benefit of 37%.

The aim of this report is to call a halt to the polarized debate on the environmental impact of GM crops and to provide a nuanced response to the many concerns that exist. Crop cultivation is by definition unnatural, and produces a negative impact on the environment. Plant breeding makes it possible to develop plants that reduce this impact. The impact, whether positive or negative, depends on the crop trait and the cultivation method, but not on the breeding technology used.

### Facts and figures

Agriculture is responsible for about 10% of the total emissions of greenhouse gases in Europe.

Worldwide, ruminants are responsible for 25% to 30% of emissions of the greenhouse gas methane. In the case of cows, the more they graze, the higher their methane production.

Had there been no genetic improvement to our agricultural crops since 1965 through plant breeding, the total surface area used for agriculture would now be between 3% and 5% greater.

Since its introduction, the cultivation of herbicide-tolerant GM crops has led to a reduction of 16.8 million metric tons in CO2 emissions and 6.3 billion liters in fuel use. That is the equivalent of taking almost 7.5 million cars off the road for a year.

A recent meta-analysis shows that the cultivation of current GM crops has led to a 37% reduction in the use of pesticides for these crops.

In 2013, the cultivation of insect-resistant GM cotton produced a saving of 21.3 million kg of insecticides (active substance).

When fields with insect-resistant GM crops are compared with conventional fields where insecticides are used, many more useful insects can be found in the fields with insect-resistant GM crops.

Both advocates and opponents of GM technology seem to agree that insect resistance can lead to a significant ecological improvement in our agricultural system.

To prevent resistance in weeds, insects, and fungi, there must be integrated pest management, which involves using several means or techniques simultaneously against a particular pest.

Before a GM crop can be cultivated commercially in the European Union, it has to undergo a risk assessment by the European Food Safety Authority (EFSA).

### Technology versus application

Many-if not all-aspects of agriculture have a negative impact on biodiversity, on soil structure and soil health, and on air and water quality. The debate on genetically modified (GM) crops raises the question of whether the cultivation of GM crops entails a greater risk to the environment than the cultivation of non-GM crops. This report addresses that question in detail. The main message is that the crop traits determine the environmental impact of a particular crop, and not the technology with which the crop was developed.

The advent of GM crops has led not only to concerns over food safety but also to questions about their potential impact on the environment. To what extent is the action of insectresistant GM crops specific? Can they also have a negative influence on useful insects or organisms? Can herbicide-tolerance genes from crops be passed on to weeds, making these more difficult to control? To what extent can GM crops have a positive effect on nature? Can they also reduce the use of plant protection products?

New crop varieties can be developed in different ways. For example, blight-resistant potatoes can be produced through conventional breeding or through GM technology (for more info, see background report "A blight resistant potato for Europe"4). The potential impact of crops, such as proliferation and effects on non-target organisms and biodiversity, is very much the same for GM and non-GM crops. However, it is natural that the impact of GM crops on the environment be investigated when these crops are cultivated on a large scale in the field. After all, with GM technology, traits can be obtained that were previously not present in crops. These new traits may have a direct effect on the environment or there may be an indirect effect on the environment due to the different method of cultivating the new crop.

Just as a GM crop has to undergo a rigorous food safety procedure prior to being approved for use in food and feed (see the report "Food safety of genetically modified crops"<sup>1</sup>), a GM crop is only allowed in the field after undergoing an in-depth environmental risk assessment. Among the aspects studied is whether the GM crop has a different effect from the non-GM crop on the soil, soil life, insects, neighboring plants, etc. The potential environmental effects of GM crops have already been the subject of scientific study for 30 years. As with the food safety tests, environmental safety analyses must be carried out on the basis of the crop traits. Clearly, no general conclusions can be drawn for all GM crops and each GM crop must be studied individually, on the basis of its trait. The GM crops in our fields today are primarily herbicide-tolerant and/or insect-resistant (see box "Current GM applications").

Following on from the background report on the food safety of genetically modified crops, this report discusses the effects of current GM crops on the environment. We also wish to offer a nuanced response, based on recent scientific literature, to the many concerns raised. To put the environmental safety of GM crops into perspective, we first discuss what impact agriculture in general has on the environment. The question of how GM crops should be evaluated is fundamental to this discussion. Should the environmental impact of a GM crop be compared with conventional cultivation, organic farming, or an integrated agricultural policy?

### CURRENT GM APPLICATIONS

The primary GM applications on the market today are herbicide tolerance, insect resistance, virus resistance, and drought tolerance. Many other applications that can contribute to sustainable agriculture (for example, blight-resistant GM potatoes) or that can be used for humanitarian purposes (for example, golden rice) are under development or pending approval for cultivation.

Herbicide-tolerant plants survive being sprayed with herbicides (weedkillers), while all weeds in the field die off. Herbicide tolerance is the subject of criticism because it promotes the use of herbicides in agriculture. However, herbicide tolerance is nothing new: all crops are tolerant to one or more herbicides. As a result, grasses can be eliminated from a potato field using specific herbicides (such as the active substances propaquizafop and rimsulfuron) because potatoes are tolerant to these products.<sup>5,6</sup>. In addition to naturally occurring tolerance, herbicide tolerance can be obtained through breeding. Plants that are tolerant to broad-spectrum herbicides have been developed through conventional breeding techniques since the 1970s. This trait gives farmers a great advantage because it allows flexible and simple weed control. In erosion-sensitive areas, herbicide tolerance also provides an indirect environmental advantage, by enabling no-till farming. This improves soil structure, causes less soil erosion, and reduces fuel use, which in turn lowers CO<sub>2</sub> emissions. Herbicide tolerance can also be developed with modern breeding techniques (such as GM technology). Herbicide tolerance is therefore not specific to any particular breeding method and primarily serves to respond to the needs of farmers. The most well-known and commercially successful examples of GM traits are glyphosate and glufosinate tolerance (marketed under the commercial names RoundUp Ready and LibertyLink respectively).<sup>7</sup> These GM crops get their herbicide tolerance from the production of bacterial proteins.

Insect-resistant crops can defend themselves against certain harmful insects. This can drastically reduce the use of insecticides, which has a positive effect on useful insects and on the environment in general. GM crops can obtain the insect resistance trait through the insertion of genetic information from bacteria, and more specifically from Bacillus thuringiensis (abbreviated as Bt). Insect-resistant Bt crops produce proteins from the Bacillus bacteria, which allows them to defend themselves from specific insects.

GMOs resistant to certain plant viruses have existed since the 1990s. The virus-resistant GM papaya is one of the major success stories. The GM papaya was developed by two American universities with no financial backing from industry and in 1998 it rescued the deeply afflicted Hawaiian papaya industry from the brink of collapse.<sup>8</sup> In addition to papayas, GM tomatoes, GM pumpkins, and GM peppers are also cultivated on a small scale.<sup>7</sup> They produce one or more proteins stemming from plant viruses or specific RNAi molecules, which makes them immune to these specific viruses (for more information, see the background report "Virus-resistant papaya in Hawaii")<sup>8</sup>.

Since 2013, GM maize with enhanced tolerance to periods of drought, has also been cultivated in the United States. Drought tolerance does not mean that the plants can grow in dry areas but it does mean that they can make it through periods of drought with no disastrous consequences for yields. The drought-tolerant maize currently available uses a protein from the soil bacterium Bacillus subtilis and, under drought stress, delivers an average of 7% more yield in comparison with conventional maize.<sup>9</sup>

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### 2 Impact of agriculture on the environment

The production of food for human and animal consumption is one of the human activities that has an enormous impact on the environment. After all, agriculture means protecting crops and keeping undesired organisms, such as weeds and insects, under control. Good soil fertility and structure are also crucial for a good harvest. This is why land is fertilized, and why tillage is used to turn over crop residues and loosen up the soil. All of these processes have an impact on the environment.

### Impact on biodiversity

Biodiversity means the variety in the type and number of living creatures present in a particular place. Humans began to farm in order to produce more food and be less dependent on hunting and gathering.

Farming entails taking over natural habitats and deforestation, which has a great impact on natural biodiversity. Food production puts even more pressure on the natural balance. After all, plants form the basis of every food pyramid, not only that of humans. Plants get attacked by bacteria, viruses, fungi, insects, and planteating vertebrates. In addition, the fertile grounds where crops are cultivated are also a favorite breeding ground for weeds or undesired plants, which compete with the planted crop. In other words, producing sufficient food for humans and animals goes hand in hand with keeping those other living creatures under control. When these are removed from the field or in many cases eliminated, this puts pressure on biodiversity both directly and indirectly. An example of a direct effect is the declining presence of insects and seed-producing weeds when fields are treated with insecticides and/or herbicides. Indirectly, however, the biodiversity of birds can also come under threat because insects and weeds form part of certain birds' diets.

The effect of pesticides is clear. But there are other farming practices that also have a substantial impact on biodiversity. Soil is turned over several times a year through tillage, while industrial tillers and heavy-duty tractors compact the deeper layers of earth. All of this affects soil life.<sup>10</sup> Spreading manure or fertilizers results in a high concentration of nitrogen



and phosphates in the field, which affects soil life and groundwater. In drylands, irrigation ensures that weeds that thrive in dry conditions have less chance to grow. Farming in itself (conventional or organic) therefore has a considerable impact on biodiversity. But this impact isn't always negative. Cultivating crops produces new ecosystems that can enrich biodiversity.

#### Pesticides

The use of the first plant protection products or pesticides dates from around 2000 BC. In ancient Sumer and China, both plant products and substances containing sulfur were used to protect harvests.<sup>11</sup> The 20<sup>th</sup> century brought great change. Extensive advances in the chemical industry paved the way for the development of many products to kill weeds, fungi, and insects. This led to a spectacular increase in yields.

Killing off organisms in the field to be able to guarantee harvests has an impact on the environment, which in many cases is tolerated. After all, the idea is to produce food for humans and animals and not for insects and fungi. When a strategy of this kind is used, the action of the plant protection products must also be as specific as possible. In this context, the terms "target and non-target organisms" are generally used. Target organisms may, for example, be insects that hamper plant growth or damage the harvest. Insects that cause no damage to the crops and useful insects that protect the crops by preying on harmful insects are called non-target organisms. In other words, the use of plant protection products should leave these insects undisturbed. Pesticides should eliminate as many targets as possible while having as little effect as possible—or ideally no effect on non-targets. However, all plant protection methods, both chemical and organic, have difficulty combating pests without undesired side effects. For example, pyrethrin, an organic pesticide approved for use in organic farming to combat insect infestation, is also poisonous to bees and other useful insects.<sup>2,12</sup>



### Fertilization

Plants have a unique ability to convert the sun's energy, water, and CO<sub>2</sub> into oxygen and sugars. This process is called photosynthesis. However, plants need more than just sugars to be able to grow well. They absorb all sorts of elements such as nitrogen, phosphorus, and potassium from the ground in which they grow. To keep plant productivity high, agricultural soil is enriched with these elements through fertilization. Manure and organic fertilizers are not consistent in composition and are not perfectly suited to the needs of a specific crop. Plant growth is limited by the element present in the lowest concentration. This means that not everything in the fertilizer is used by the plant, resulting in ground and surface water being contaminated with extra nutrients. In addition, manure acidifies the soil. Sometimes, artificial fertilizers are a better option from an agricultural point of view,

because their composition can be optimized for the crop. However, producing artificial fertilizers uses a lot of energy and pollutes the environment, in addition to having a high cost price.

Fertilization of agricultural land is one of the main causes of contamination of surface waters. For example, phosphate fertilization washing into streams and rivers can lead to eutrophication of coastal waters and waterways.<sup>13,14</sup> Eutrophication is an explosive increase in algae growth, resulting in all oxygen, and therefore all forms of life, disappearing from the water ecosystem.

### Tillage

We are so familiar with certain agricultural practices, such as tillage, fertilization and preparation of fields for sowing, that we assume them to cause little harm to the environment. However, these actions are responsible for soil erosion and the loss of farming productivity. Depending on the type of soil and climate, tillage can have varying side effects. Loosening soil is necessary to make a heavy and compacted soil airy, but it can be detrimental to erosion-sensitive and



light soils. Tillage, or turning over of the soil, crumbles the ground, which means that fertile ground can more easily fly away with the wind or wash away after a heavy rain shower. Turning the ground also brings moist earth to the top, drying it out. In areas with wet winters this is good, because sowing can occur earlier. It needs to be avoided in dry areas, however, because the additional evaporation resulting from tillage will compromise the moisture reserves in the soil. In addition, the time at which tillage occurs and the condition of the field can play a major role. After all, driving over wet soil with heavyduty tractors can compact the ground completely. Tire tracks and the ensuing puddles, which can be seen on Flemish fields from harvest time onward, are a sign of compressed soils. This makes it more difficult for roots to develop, and this inevitably has a negative effect on yields.

In Europe, the deterioration of soils through erosion (especially in southern Europe) and soil compaction (in the wetter regions) are some of the greatest problems caused by farming activities.<sup>15,16</sup> This is why, in recent decades, increasing emphasis has been placed on no-till farming.



By not turning over the ground or mixing less intensively, more crop residues remain on the field. As a result, soil binds together more, which protects it more effectively from erosion. Keeping soil processing to a minimum also results in an optimal soil structure. The bearing capacity of soil is thereby increased, meaning that under wet conditions a no-till field is easier to ride on than a tilled field.<sup>16-18</sup> Additionally, there is far less disruption to soil life, which has a positive effect on the biological activity in the soil.<sup>19-23</sup> The presence of crop residue also attracts more birds and small animals. Spontaneous decomposition of residue creates new ecosystems, which increases biodiversity in the field.<sup>24</sup>

So if there are many benefits to no-till, why do we still till? As always, there is a flip side. Where disturbance of the soil is kept to a minimum, there are also more weeds. And weeds are one of a farmer's worst nightmares. One of the strategies for getting rid of weeds before crops are planted is to turn them under. However, if the aim of no-till farming is to preserve the soil structure, weeds need to be eliminated in another way. One way—using herbicides—can also have a negative effect on the environment and on biodiversity.

### Air pollution

Human activity has caused a dramatic increase in the emission of greenhouse gases such as carbon dioxide ( $CO_2$ ), nitrous oxide ( $N_2O$ ), and methane ( $CH_4$ ) over the last 200 years. Farming plays a major role in this. In Europe, agriculture is responsible for about 10% of the total emissions of greenhouse gases.<sup>25</sup> And it is not only agricultural vehicles that pollute the air. As an example, rice fields are responsible for 10% of the worldwide human-related emissions of methane, a greenhouse gas that contributes to global warming.<sup>26</sup> Livestock also has a significant impact. In the United States and Europe, ruminants are responsible for 25% to 30% of methane emissions.<sup>6,27</sup> Methane is produced in the stomachs of cattle as a byproduct of microbial digestion and is primarily emitted through respiration. Methane production is mostly determined by type of fodder. In the case of cows, the more they graze, the higher their methane production.

#### Irrigation

During growth, crops are confronted with an array of pathogens, which have a negative

effect on harvests. Lower yields from plants as a result of external factors are often the conseguence of "stress". When living organisms such as insects and bacteria are the cause, this is referred to as biotic stress. But there is also abiotic stress, which refers to reduced growth as a result of suboptimal environmental conditions—such as a lack of nitrogen in the soil. A lack of certain nutrients in the soil can be remedied with suitable fertilization. Reduced yields as a result of drought, another abiotic stress factor, can be combated with irrigation. 40% of food production comes from irrigation farming.<sup>7</sup> The water used for this often comes from underground freshwater reserves. Around 70% of water extracted from nature is used for farming.<sup>28</sup> And the prospect of our freshwater reserves becoming exhausted is certainly not good news.

# **3** Direct effects of a (GM) crop on the environment

Besides standard farming practices, the cultivation of a certain crop can have additional implications for the environment as a result of its specific traits. The direct effects of specific crop traits are discussed in this chapter. Such effects can be either positive or harmful for the environment and are not determined by the breeding method used to develop the crop.

### Use of plant protection products

Every year, 2.4 million metric tons of pesticides (such as insecticides, fungicides, and herbicides) are used.<sup>29,30</sup> However, in the plant world, disease is the exception rather than the rule. With evolution, plants have built up immunity to a great number of attackers such as fungi, viruses, bacteria, and insects. However, not all disease-resistant plants are suitable for consumption or popular as commercial varieties. The consumer and processing industry have their own criteria that a food product must meet: taste, size and shelf life, to name but a few. A good example is the Bintje potato. Bintje is highly susceptible to potato blight, as a result of which it cannot be cultivated on a large scale without the use of substantial quantities



of fungicides. Nevertheless, although varieties exist that require less use of pesticides, Bintje continues to account for around 50% of the area used for production of potatoes in Belgium. In the case of potatoes, as well as other crops, breedersinvestagreat deal of time and resources on transferring disease resistance that occurs in nature to plant varieties of commercial interest, in order to increase harvest security and reduce the use of pesticides in agriculture. In organic crop breeding, efforts are also made to breed varieties in such a way as to develop wide foliage very soon after germination. This gives germinating weed seeds less light and reduces the need to control weeds.

Alongside fungi and weeds, insects are also a big problem in agriculture. Over the course of evolution, plants have developed a range of strategies to arm themselves against insects and other plant-eaters. These range from morphological changes such as thorns to the production of specific defense substances. These defense substances are often proteins and secondary metabolites such as lectins, cysteine protease inhibitors, glucosinolates, and alkaloids.<sup>31</sup> Secondary metabolites are chemical substances that play a significant role in the protection and survival of plants. Defense substances form part of the biological warfare between plants and attackers. Some substances are continually produced by the plant, while others are only produced when the plant is attacked.

Lectins are sugar-binding proteins, which abound in the plant kingdom.<sup>32</sup> Usually, a plant only produces lectins when it is actually being



attacked. Certain varieties of wheat produce lectins in their leaves only in the case of an insect attack.<sup>32</sup> There are hundreds of different types of lectins that protect plants against plant-eaters, and some lectins—such as the phytohemagglutinin in kidney beans—are even poisonous to humans. This is why these beans need to be cooked for a long time to break down the lectin before they can be safely consumed. Commercial plant varieties can, in some cases, be made resistant to certain insects with the help of cross-breeding as long as there is a crossable plant available that produces lectins or another protective product.<sup>31,33</sup> The cultivation of an insect-resistant crop of this kind will lead to a reduced use of insecticides, which is positive for the environment, but the protective substance such as lectin can also be directly or indirectly detrimental to useful insects or other non-harmful organisms, which is negative for biodiversity. The impact of the insect-resistant crop on useful organisms is determined by the specific characteristics of the resistance mechanism (for example, lectins) and not by the technology (cross-breeding, GM technology, etc.) with which the resistance mechanism has been introduced into the plant. In addition to making use of traditional breeding techniques such as cross-breeding, insect resistance can also be obtained with the help of GM technology. The most famous examples of this are undoubtedly Bt crops. These crops have received hereditary information from the bacteria *Bacillus thuringiensis*, as a result of which the plant produces one or more proteins that are harmful to certain insects.<sup>34</sup>

Insect-resistant crops are plants that protect themselves against one or more groups of insects. In the case of genetically modified Bt crops, the resistance targets caterpillars and larvae of moths or beetles. As a result, Bt crops no longer need to be treated with the chemical or biological methods that are normally used to tackle these insects. The insect-killing effect of Bt has already been known for over 100 years and commercial products based on Bt have been available since the 1940s.<sup>34</sup> Bt sprays account for more than 90% of the organic pesticide market and are an important method for keeping insects under control, especially in organic farming.<sup>35,36</sup> Nowadays, there are four commercially bred crops that have received, through GM technology, the genetic information to produce Bt proteins: cotton, maize, soy, and eggplant. This makes for a drastic reduction in the use of insecticides, particularly in cotton farming.<sup>34</sup> Cotton is a crop that requires copious amounts of insecticides, especially against the bollworm. Cotton is cultivated on 2.5% of the global arable land but is responsible for 16% of worldwide insecticide use.<sup>37</sup> In 2013, 21.3 million kg of insecticides (active substance) were saved as a result of the cultivation of Bt cotton.<sup>3</sup> This comes to about half of the total insecticide use in cotton farming, a reduction of 48.3%, to be exact. If we consider cultivation since 1996, this

makes a total of 230 million kg of insecticides not used thanks to the cultivation of insectresistant GM cotton.<sup>3</sup> In addition, large-scale cultivation of disease-resistant GM crops results in a reduction of the overall disease burden, which then leads to a reduction in the use of pesticides in neighboring non-GM crops.<sup>38</sup>

For maize and soy cultivation, the quantities of insecticides spared are less spectacular because the insects for which these Bt crops are developed are less dominant than the bollworm in cotton farming. This does not mean that there are no significant ecological advantages, however. In 2013, 8.2 million kg of insecticides were saved as a result of Bt maize cultivation, a drop of 84% in the use of insecticides specifically targeting stem and root borers.<sup>3</sup> In the period from 1996 to 2013, Bt maize cultivation caused a reduction of 72 million kg in insecticides, which means that the environmental impact of GM maize cultivation is 53% lower for this period than that of conventional cultivation. Thanks to the limited Bt soy cultivation, which started as late as 2013 in South America, a saving of 0.4 million kg of insecticides was made, or 1% of total insecticide use in soy farming.<sup>3</sup>

The positive effect of the cultivation of Bt crops has been independently confirmed by agricultural economist Charles Benbrook,<sup>39</sup> a critical voice in the GM debate who insists, against all scientific data, that there is no scientific consensus on the safety of GM technology.<sup>40</sup> So both proponents and opponents of GMO seem to agree that insect resistance can lead to significant ecological improvement of our agricultural system and that Bt crops have convincingly proven this over the last few decades.



### The honey bee and other non-target organisms

When plants that protect themselves against pest insects are cultivated, it is crucial to look into their effects on useful insects or organisms that do not harm the crops (non-target organisms). The intention, after all, is not to cultivate a new crop, variety, or trait that causes adverse changes to the ecosystem. This is not always straightforward, however-especially when a non-target organism has great physiological similarities with a pest that must be controlled. Being able to eliminate such pests without causing an undesired effect on the non-target organisms is the great challenge for all crop protection methods, whether chemical or biological. Even protection that has evolved naturally is not always selective. Plants produce certain protective substances to arm themselves against pathogens and/or plant-eaters. The aforementioned lectins, (see page 15), which are not necessarily very specific, are a perfect example.

In the debate on the effect of agriculture on non-target organisms, the honey bee garners the most attention. This useful insect not only produces honey but is responsible for the pollination of a guarter of all crops.<sup>41</sup> Good pollination by insects is crucial for many crops, in terms of both quality and quantity of harvests. Beekeepers all over the world are witnessing dwindling bee populations and often erroneously perceive the introduction of GM crops as being partly to blame. However, in northern Europe (for example, Belgium), the decline in bee populations is just as serious and we are seeing abnormally high death rates among honey bees.<sup>42</sup> This is occurring despite there not being a single GM crop cultivated commercially in Belgium or neighboring countries. It is therefore clear that GM crops cannot be the cause of the dwindling bee populations.

These theories primarily target insect-resistant Bt crops. Bees are insects, after all, and in the course of their pollination activity they inevitably come into contact with large quantities of pollen where Bt proteins are present. The suggested link is not supported by scientific data, however. In the first place, this is because Bt proteins have a highly specific mechanism (for more information, see the background report "Bt cotton in India"34). Once consumed, a Bt protein in the gut of a susceptible insect is recognized by specific intestinal wall receptors: a type of antennae that trigger a reaction when they receive a signal.<sup>34</sup> Depending on the type of Bt protein, Bt only works against specific insect families that have the right intestinal "antennae". The Bt proteins used today in agriculture are not recognized by bees, which means that bees cannot, by definition, be susceptible to the Bt crops approved today. This has been confirmed by a large number of studies in the laboratory and in the field.43-47 These studies have shown

that in comparison with pollen from non-Bt crops, the pollen from Bt crops has no effect on the bees' weight, orientation, quantity of pollen gathered, activity of the pollen gatherers, health of the colony, or weight or development of the brood.43-47 It was also demonstrated that Bt proteins consumed had no effect on the bees' intestinal cells.48

The effect of Bt proteins was not only studied for bees. Other insects were also studied. In Spain, for example, studies were carried out to determine the effect of Bt maize on different insects in the field. A meta-analysis based on 13 different Spanish field tests demonstrated that the 26 insect groups studied suffered no ill effects from Bt maize.<sup>49</sup> There are also studies that show that Bt crops can sometimes have a negative effect on the development of certain predators (insects that feed on other insects) and parasitoids (insects that lay their eggs in the body of other insects) of Bt-sensitive

### THE MONARCH BUTTERFLY

One of the most famous studies on the effect of GM crops on non-target organisms is the one on the monarch butterfly in the United States. In 1999, three entomologists from Cornell University in New York stated in the authoritative journal Nature that the pollen of insect-resistant Bt maize was harmful to the monarch butterfly.<sup>51</sup> That article sparked considerable debate. It made front-page news and spread throughout the world. The monarch butterfly, just like the honey bee, is an insect that many people have a soft spot for. In North America, the monarch butterfly has symbolic meaning and is often referred to as "the Bambi of the insect world." 52 They are not only incredibly beautiful, with their black-veined orange wings, but they also have a mysterious side to them. Every year, they set off on a heroic journey from Canada and the United States to the forests of Mexico, where they spend their

insects.<sup>50</sup> These effects are often attributable to an indirect ecological effect resulting from the reduced presence of Bt-sensitive insects, which is covered in the following chapter (see page 39). The sporadic and limited negative effect of Bt crops on non-target organisms and the environment is negligibly small in comparison with the impact of traditional insecticide use.<sup>50</sup>



winters. This annual migration of many millions of butterflies at the same time over remarkable distances is one of the most famed phenomena in nature.

During their great journeys, monarch butterflies lay their eggs on a plant known as the butterfly flower (Asclepias syriaca), which comes from the periwinkle family common in North America and Mexico and is labeled a weed. The larvae only eat the leaves of the butterfly flower. They feed on the leaves for two weeks, then pupate and turn into a butterfly. The butterfly flower is therefore crucial to the existence of the monarch butterfly. The researchers involved in the 1999 Nature study fed leaves sprinkled with the pollen of insect-resistant Bt maize to monarch butterfly larvae. The researchers identified that 44% of the larvae died and that those that survived were smaller than the larvae that ate the leaves without the Bt pollen.<sup>51</sup> The fact that the larvae of butterflies were sensitive to some Bt proteins was in itself not surprising news. Monarch butterfly larvae and the moth larvae that Bt crops are designed to tackle both belong to the Lepidoptera order of insects after all. In addition, Bt proteins had already been used as an organic pesticide since the middle of the 20th century and it was already known that spraying affected the population of Lepidoptera.<sup>53,54</sup>

However, the big question left unanswered in the Nature study was whether the larvae could also actually come into contact with Bt proteins in nature. Another important question is whether they are sensitive to all Bt proteins or only to one or more specific Bt proteins. The Nature study used pollen that only produced Cry1Ab proteins.

Butterfly flowers grow in and around maize fields. In a field they can be sprinkled with relatively large quantities of pollen during flowering, but then again in a field, they are seen as a weed, so farmers get rid of them as much as they can. Outside the field, the plants must be sufficiently close to the maize in order to be covered in maize pollen. Additionally, the maize pollen must fall on the leaves of the butterfly flower precisely at the time that the butterflies lay their eggs and the larvae emerge. To ascertain whether there is a danger to monarch butterflies under natural circumstances, the US Department of Agriculture and several universities carried out large-scale research.

The various studies were published in September 2001 in a joint edition of Proceedings of the National Academy of Sciences USA (PNAS) and concluded that the risk to the monarch butterfly was insignificantly small.<sup>54-59</sup> In the original Nature study, unrealistically high quantities of Bt pollen had been used. Most Bt crop varieties produce too few Bt proteins in the pollen to be harmful to butterflies in the field. The concentration of Bt proteins differs between Bt crop varieties, however. One variety (Bt176) produced far more Bt proteins in the pollen than other maize varieties (because the Bt gene was under the control of a pollen-specific promoter). In the case of Bt176, it was possible to attain a Bt concentration harmful to the monarch butterfly on the butterfly flowers.<sup>55</sup> However, the Bt176 maize variety was a variety that performed less well in the field and, at the turn of the century, occupied only 2% of the area used for the production of maize in the United States. The high Bt concentration in the pollen was a decisive factor in stopping the sale of this largely unsuccessful variety.<sup>52</sup> Another important observation is that the peak of maize pollen production in the southern test areas did not coincide with the presence of larvae on the butterfly flowers. It was only in the northern areas that there was an overlap in half the cases. About 50% of all larvae counted during the test

period were present during the flowering of the maize plants.<sup>56</sup> This may sound like a lot, but the butterfly flowers on which monarch butterflies lay their eggs do not only grow in or around maize fields. In simulations, it was ascertained that this only occurred in 56% of cases.<sup>56,58</sup> When you also take into account the maximum overlap between the presence of larvae and maize flowering (62%),<sup>56</sup> the current level of adoption of Bt maize in the United States (93%)<sup>7</sup>, and the chance that the pollen of the Bt crops currently cultivated would have a disadvantageous effect on the monarch larvae (0.7%),<sup>58</sup> this means that 0.2% of monarch butterflies are in danger. This chance is insignificantly small in comparison with the risk to monarch butterflies when a maize field is sprayed with Bt products and/or other plant protection products.

The PNAS studies made it clear that risk assessment for non-target organisms is very important and that the working mechanism behind the insect-resistant crops must be as specific as possible. Under ideal circumstances, the insect-repellent substances in Bt crops should only be produced in the parts of the plants that are susceptible to insect attacks. Pollen that can be a source of food for useful insects or that could cover other plants (such as butterfly flowers) must contain as low as possible a concentration of these products. With the Bt crops currently cultivated, this is already the case. Thanks to the PNAS studies, it also became clear that the commotion surrounding the original Nature article was disproportionate. However, the PNAS studies attracted barely any media attention at all, probably because of the 9/11 attacks in the United States.

#### Yield per surface unit

The increasing demand worldwide for farming products as food, feed, and fuel puts great pressure on the environment and on the agricultural area available. The lower the yield that can be obtained per hectare of agricultural land, the more natural vegetation must be sacrificed and converted to agricultural land. The cultivation of a low-yield crop therefore has a negative effect on the environment, which should not be underestimated. Keeping the increase in the agricultural area under control is one of the most important challenges that must be overcome in order to prevent the loss of natural vegetation and biodiversity.

Between 1965 and 2004, the world population doubled and an average of 10% more food was consumed per person.<sup>61</sup> Despite this, the agricultural area increased by only 2%. This was

primarily due to the increased yield per hectare enabled by the Green Revolution. This agricultural revolution between 1960 and 1980 was characterized by the advent of fertilizers, crop protection products, and irrigation techniques, and came at the same time as the development of plant varieties that reacted optimally to fertilizers.<sup>62</sup> The economists Evenson and Rosegrant calculated that in the year 2000 the agricultural area would have been 3% to 5% greater if no genetic improvement had been made to agricultural crops through plant breeding since 1965.63 This amounts to a saving of 9 to 12 million hectares of agricultural area in developed countries and 15 to 20 million hectares in developing countries. This total saving of 24 to 32 million hectares through technological breakthroughs between 1965 and 2000 more or less ties in with the recent data from James Stevenson from the



FAO (Food and Agriculture Organization) and American colleagues.<sup>61</sup> On the basis of a model that takes into account more parameters, they calculated that in 2004, between 18 and 27 million additional hectares agricultural land would have been in use if the agricultural crops were to have the same yield as in 1965. Of this figure, an estimated 12 to 18 million hectares of land was spared in developing countries and 2 million hectares of deforestation prevented.

These calculations show that the increased yield per hectare led to a smaller increase in the agricultural area. Local socioeconomic aspects can distort these figures, however. Increased productivity is necessary to combat the increase in agricultural area and deforestation but it is not sufficient. Higher productivity per hectare makes agricultural activities more financially efficient, which in itself can stimulate the extension of the agricultural area. This higher productivity per hectare can also reduce the price, which can boost demand and lead to more production. New technologies can therefore, paradoxically, also contribute to an increase in the agricultural area. This is why scientists from the United Kingdom and Brazil recently called for action from the government.<sup>64</sup> This action can include economic sanctions (taxes, subsidies), reservation of nature areas in the agricultural area, and the issuing of certificates.<sup>64</sup> Although high-yield crops are very much necessary, better monitoringcombined with a well-thought-out agricultural policy from the local governments—remains essential in order to tackle the increase in the agricultural area.



### Pollen-mediated gene flow (vertical gene transfer)

The chance of cross-breeding occurring between crops and wild varieties has always existed and the flow of genes from cultivated crops to wild crossable varieties has been occurring since the beginning of agriculture. There are various factors that determine the likelihood of genes being spread via pollen<sup>65</sup>:

- The way in which pollen is transferred (wind, insects), the distance between plants, and the life cycle of pollen.
- Synchronization of flowering between plants.

- The sexual compatibility or the possibility of two plants producing fertile offspring.
- The ecology of the wild population and the extent to which the gene obtained can offer a selective advantage for the wild varieties.

The technology used to breed the crops (cross-breeding, mutation breeding, GM technology) has no effect whatsoever on the chance or the impact of gene flow in nature. This impact is entirely determined by the genes and therefore the traits for which the transferred genes code.

Take for example the cultivation of golden rice (a GM rice variety that produces provitamin A in the grain), the cultivation of a non-GM soy variety that is herbicide-tolerant, and the cultivation of an insect-resistant non-GM maize. The last two crops in this example were developed through classic breeding techniques and not through GM technology. The chance of provitamin-A production in GM rice being transferred to wild rice varieties is just as great as the chance of herbicide tolerance in non-GM soy spreading to neighboring plants that can be cross-bred with soy. The same applies to the insect resistance trait. The impact, however, is very different. In the case of GM rice, neighboring plants would be able to inherit the ability to produce provitamin A, a trait that is currently prevalent in nature and which does not create a selective advantage for the plant. In the case of non-GM soy, neighboring plants can inherit tolerance to certain herbicides, as a result of which these herbicides can no longer be used to eliminate them. This advantage is only valid for plants in an area in which the herbicide is used. In an area in which the herbicide is not

used, wild plants have no advantage in developing herbicide tolerance. This is different in the case of insect resistance. If this trait is introduced in wild variants, these plants are better armed—even outside an agricultural environment—against pest insects, which gives them an advantage and can have an effect on diversity.

In other words, gene flow in nature only has an impact on the environment if the gene can offer a selective advantage for plants that can cross-breed with the planted crop. Wild plants that obtain a selective advantage in this way can cause disruption to the existing ecological balance and suppress other plants. As a result, the choice of trait is an important aspect of the risk assessment previous to an approval for cultivation. However, remarkably enough, from a regulatory point of view this appears to only be necessary for GM crops, even though the example above demonstrates that the breeding technology is of minor importance compared with the crop trait. The transfer of genes in a population has no impact on the environment as long as these genes do not disturb the existing ecological balance.

From a socioeconomic point of view, gene flow must be evaluated differently, especially in the case of GM crops. Farmers, the food industry, and consumers want to make a conscious choice in certain cases between different production types. As a result, GM crops are allowed in conventional agriculture but not in organic agriculture. To respect freedom of choice and to eliminate financial damage in the organic sector (loss of organic label), there are directives laid down to limit gene flow from GM fields to conventional or organic cultivation





fields to a minimum. Legally speaking, an unintended admixture of GM traits of less than 0.9% in organic products does not lead to the loss of an organic label, but in practice, the organic sector generally has a zero-tolerance attitude to GMOs.

The fact that co-existence of GMOs and non-GMOs is possible in practice provided that there is limited tolerance, both in cultivation and in the supply chain, is demonstrated by the EU-financed PRICE project. PRICE is the acronym for PRactical Implementation of Co-Existence in Europe and is the project that investigated the effect of the existing co-existence measures in Europe as regards gene flow of GM maize to neighboring fields growing non-GM maize. There are several measures that appear suitable for eliminating a significant admixture of GM and non-GM products.<sup>66</sup> In Europe, the tolerance threshold is set at 0.9%. This threshold of 0.9% is important because when more than 0.9% of an ingredient in a product originates from a GM crop, the product must be labeled in Europe as a product that contains GMOs.

To eradicate gene flow via pollen, minimum distances between GM and non-GM fields must be adhered to. These distances differ from crop to crop. In crops such as potatoes, which produce little to no pollen and propagate via bulbs, a distance of 5 meters proves to be sufficient.<sup>4</sup> For maize, a recent study in Mexico (the birthplace of maize) showed that



with a minimum distance of 20 meters, the gene flow between GM and non-GM was able to be kept under the 0.9% threshold.<sup>67</sup> In Flanders investigations were also carried out to determine whether GM maize cultivation could be compatible with conventional and organic maize cultivation.<sup>68</sup> The conclusion of the ILVO [Flanders Institute for Agricultural and Fisheries Research] was that the separation distance of 50 meters previously established by the Flemish government was more than sufficient to keep GM contamination of the conventional maize crops by flying pollen under the 0.9% threshold. The PRICE project showed, with the help of field

### THE CHAPELA AFFAIR

In October and November of the year 2000, Ignacio Chapela and his graduate student David Quist collected corncobs from landraces in Oaxaca, Mexico.<sup>70</sup> The maize landraces (local maize varieties) are conserved and bred by local farmers and growers, who cultivate them in small fields year after year. The varieties have evolved genetically over the years through human intervention and environmental conditions. The landraces are a great source of diversity and are cherished by the local population. To maintain the high level of diversity, it is recommended that these varieties are not cultivated together with commercial varieties. Certain strong crop traits of commercial varieties could appear in landraces as a result of cross-breeding, resulting in some varieties being able to obtain a selective advantage over other landraces. Despite this care and because GM technology has so far only been used to improve commercial varieties, Mexico does not allow the cultivation of current GM maize. It therefore came as a great surprise when Chapela and Quist reported in the highly regarded journal Nature in 2001 that they had found DNA fragments of GM maize in the DNA of four of the six maize landraces tested.<sup>70</sup>

tests, that when a GM field and a non-GM field are closer than 20 meters to each other, gene flow can still be prevented by placing a buffer zone between the GM and non-GM maize.<sup>69</sup> A zone of 12 rows of non-GM maize appears to be sufficient. As an alternative, the GM and non-GM maize can be sowed at different times. If there are four weeks during the month of April between the sowing of GM and non-GM maize or two weeks in the month of May, a GM crop can even be cultivated right next to a non-GM maize field with no chance of pollen-mediated gene flow.<sup>69</sup>

In other words, it is possible to prevent gene flow in the field. However, to obtain complete separation between GM and non-GM products, additional measures must be taken. Cleaning harvesting machines and correctly labeling the harvest are the most important aspects. These extra measures to guarantee freedom of choice also entail additional costs. Ecologists and environmental activists reacted furiously to this because, in their minds, this was proof that GM crops could "contaminate" other plants, resulting in the disappearance of local varieties. Scientists were not surprised at the results. Sexual reproduction is one of the most natural phenomena in existence. For as long as maize has existed, maize plants have exchanged genes via pollen. There is no reason what-soever for fertile GM crops to behave differently. DNA from commercial varieties can certainly be found in the landraces and DNA from landraces in the commercial varieties, although the latter is much harder to prove. Mexico has never given permission for the commercial cultivation of GM maize, but this does not mean that all farmers follow this prohibition. Mexico imports great quantities of GM maize for use in food every year. GM maize seeds are therefore widely available in Mexico and some farmers are known to use these seeds to sow a new crop, or mix these seeds with seeds they have harvested themselves if they do not have enough seeds to sow.<sup>71</sup> The cultivation of GM maize plants in the vicinity of or between landraces could therefore have led to one or more seeds falling into the cobs of the landraces, resulting in them containing DNA fragments from GM maize. In any case, these extra DNA fragments would not simply result in the disappearance of the landraces. In fact, DNA fragments from the landraces may also appear in one or more seeds of the GM maize plants.

Furthermore, Chapela and Quist's data was not entirely clear. The authors had analyzed all maize seeds from one cob at once and only received a weak signal indicating the presence of GM material. In subsequent years, other labs repeated the experiment set up by Chapela and Quist and obtained different results. In one study, 870 plants in 125 Mexican fields in 18 different locations were tested. DNA from a GM crop was not detected in a single one of the 153,746 seeds tested.<sup>72</sup> In 2009, another study appeared that did show DNA from GM crops in Mexican landraces, although the frequency was much lower.<sup>73</sup>

The latest study therefore confirmed Chapela and Quist's observation that DNA material from GM maize can be present in Mexican maize varieties. However, there was another reason for the commotion generated by the publication of the Nature article. Scientists were surprised and unsettled by two other claims made by Chapela and Quist. In the Nature publication, the authors stated that introgression had occurred.<sup>70</sup> This term refers to a phenomenon in which the DNA of one plant (for example, a GM plant) is stably built into the DNA of another plant (for example, a landrace) through repeated backcrossing over several years, bringing a new intermediate hybrid or variety into existence. During an introgression process of this kind, more and more DNA from one parent plant (for example, GM maize) remains in the new hybrid and less and less DNA from the other parent plant (for example, the local maize variety). In other words, if efforts are not made to preserve the local varieties, genes from local varieties could disappear from a population. Chapela and Quist had no data at all to support this introgression hypothesis. The analysis of the local maize cobs generated only a weak signal indicating the presence of GM material was received. The GM maize that they had used as a positive control in the experiment gave a very strong signal. This indicates that in the cobs of the landraces, only a very limited number of seeds were produced after pollination with GM pollen. To find out whether introgression had occurred, Chapela and Quist should have first analyzed leaf material from landraces and not seeds. Chapela and Quist should also have planted the "positive" seeds and, in the following generation, analyzed whether cross-breeding had in fact occurred between GM plants and landraces. These experiments were not carried out. The major irritation that could be detected in the responses from other scientists to the

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Nature article was therefore also primarily in response to the sloppy research by Chapela and Quist, who had not carried out simple control experiments.<sup>74,75</sup>

A second conspicuous error in the conclusions of Chapela and Quist was that according to the authors, the DNA fragments of the GM crops behaved in a different way in the DNA of landraces. Certain DNA fragments should, according to them, move uncontrollably in the DNA of maize landraces. This suggestion goes completely against the grain of all the knowledge built up over several decades of molecular research and could rely on very little acceptance from the scientific community. Chapela and Quist based this on results obtained through the inverse PCR technique, a method highly susceptible to producing erroneous results.<sup>74,75</sup> This technique is useful on the condition that the results are verified afterwards to confirm or refute them. Control experiments were not conducted by Chapela and Quist and their results can very easily be explained by the disadvantage of the inverse PCR technique: the creation of false results.<sup>74,75</sup> Chapela and Quist's publication is a lamentable example of badly conducted research, which managed to slip through the net of the normally very strict quality control of journals such as Nature and ended up being published. Fortunately, the publication of such articles brings fellow scientists out in force to put the flawed hypotheses right.

### Horizontal gene transfer

The spread of pollen as described above can lead to transfer of genetic information from parents to descendants, which is also called vertical gene transfer. In addition to this, there is such a thing as horizontal gene transfer. This is the exchange of genes between organisms with no sexual reproduction involved. Bacteria are incredibly good at this.<sup>76</sup> They are exceptionally skilled at taking and exchanging DNA between themselves. But there are other organisms (such as plants, mites, and nematodes) that, over the course of evolution, have taken over genetic information from other organisms, mostly from bacteria. Horizontal gene transfer has therefore played a role in the adaptation process of organisms to changing living conditions and, in isolated cases, has even caused the emergence of new varieties.77

Horizontal gene transfer suddenly became a hot topic in the debate surrounding GM crops, as a result of the discussion on antibiotic resistance genes. These are genes that protect an organism against the harmful effect of antibiotics. These genes are being used increasingly less during the development of GM crops, because of the negative perception of antibiotic resistance genes. But at the outset of GM technology (the period from which the currently cultivated GM crops date), they were widely used. An antibiotic resistance gene was built into the DNA of plants, along with the gene of interest, so that the GM plants could be efficiently selected in the laboratory from the large group of GM and non-GM plants. The worry that these antibiotic resistance genes from GM crops could be transferred to bacteria

(or other organisms such as fungi) in the soil emerged very quickly, primarily because these genes originally come from bacteria. Dozens of studies were conducted in the laboratory and in the field. In the period between 2002 and 2012 alone, there were 59 studies.<sup>78</sup> It emerged from these that the frequency of horizontal gene transfer from plants to other organisms (including bacteria) was extremely small and that this could not yet be proven in the field.<sup>76</sup> Only under artificial laboratory conditions could transfer be observed, albeit at a very low frequency (probability between 1 in 10,000 and 1 in 100 million), between plant and bacteria of a piece of DNA that shows great similarity in plant and bacteria.79,80

In the highly improbable case that antibiotic resistance genes could nevertheless be transferred from a GM plant to a bacterium, it is important to know which antibiotic resistance genes are concerned. These genes are divided into three different groups on the basis of their use in human and veterinary medicine and depending on their presence in the environment.<sup>1</sup> In the EU, only antibiotic-resistance genes that are already widespread in the environment or genes that deliver resistance to antibiotics that are of little or no importance in medicine may be used during the development of commercial GM crops. This is overseen by the international and national institutions that regulate food and environmental safety such as the European Food Safety Authority (EFSA).

#### Invasiveness

The introduction of plants or animals into an area in which they are not yet present must always be closely monitored. After all, there are sufficient examples from the past where the intended or unintended release of certain animals or plants had a dramatic impact on the fauna and flora of a particular area. An example of this is the release of rabbits in Australia or the South American water hyacinth, which has completely overgrown Lake Victoria in Kenya since 1992. Another example is the kudzu (an Asian climbing plant) invasion of American fields and nature areas.<sup>81</sup> Kudzu was first introduced in the United States in the late 19<sup>th</sup> century to provide shade to front porches and courtyards. At the beginning of the 20th century, it was promoted as feed and planted widely to combat soil erosion of agricultural land. Because of the ideal climate conditions, the absence of disease and plagues, and the great difficulty in eliminating it, the plant has since been able to spread like wildfire. It overgrows roads and bridges, houses, telephone poles, and transmission towers, and covers and destroys local vegetation. In the south of the United States, kudzu is known as "the vine that ate the South."<sup>81</sup>

Generally speaking, the introduction of a new crop trait can induce overgrowth of plants in two ways. On one hand, the new trait can cause the crop to grow rampantly in an agricultural area, while on the other hand, a new crop trait can spread through cross-breeding to wild crop varieties.

A hypothetical example of the first situation is frost resistance in potatoes. After the potato harvest there is always a small part of the

potato that remains in the ground. Most of this will freeze to death over the winter and not form weeds the following spring. Frost-resistant potatoes that remain in the field, however, could germinate in the spring and appear as a weed in the following harvest.

A hypothetical example of the second situation is the spread of a drought-resistant gene to a wild crop variant. The descendants of this plant can, over time, spread to dryer areas where they can suppress other plants. If, for example, the suppressed plants formed a better habitat for certain insects, this may cause a disturbance of

Kudzu, a climbing plant native to Azia, overgrows local vegetation in the United States.



the ecosystem and biodiversity on many levels. To correctly estimate the risk of invasiveness, it is important to also take into account the area of origin of the crop. Maize, for example, was introduced from South America. In our region, there is no wild maize. Therefore, there is no risk whatsoever of GM maize influencing the natural maize populations in Europe. The same applies to potatoes and other crops from other continents. In countries such as the United States, Argentina, and Brazil with considerable soy plantations, again there are no wild soy varieties, because the plant originally came from Asia.

### Indirect effects of a (GM) crop on the environment through changes in cultivation practices

Alongside the direct effect of a plant characteristic on the environment, the agricultural practices that go hand in hand with the cultivation of a specific crop can also have an effect on the environment. The cultivation of herbicide-tolerant plants, for example, allows farmers to till less, which has significant positive effects on the soil structure and soil organisms. On the other hand, the misguided cultivation of purely insect-resistant crops can lead to the emergence of resistant insects.

### Transition to no-till farming

In contrast to disease-resistant crops that can provide a direct environmental advantage because of their reduced need for pesticides (see page 15), herbicide-tolerant plants depend on the use of herbicides. Nevertheless, herbicide-tolerant crops—in particular, the cultivation of glyphosate-tolerant crops—can provide a clear environmental advantage, provided that good agricultural practices are adhered to.

Crops that are tolerant to broad-spectrum herbicides such as glyphosate go hand in hand with no-till farming. This is a form of agriculture that causes little to no disturbance to the soil. Most of the time, this means that the ground is not turned but loosened. After the harvest, the ground is shred and crumbled with the help of teeth that are pulled across the ground without moving it. A more extreme form is the "direct-seeding" technique or no-till farming in the strictest sense. Here, any kind of ground processing is eliminated, as a result of which the soil is always covered with a crop or crop residues from previous cultivations. Only a small groove is made in the soil to sow the crop, leaving the soil completely undisturbed.

There are a number of significant environmental advantages linked to no-till farming (see page 11). First and foremost, it is beneficial for the health of the soil, the soil life is disturbed far less, the likelihood of erosion is reduced, and the bearing capacity of the soil is increased. A further advantage of no-till farming is the reduced use of machine tillage. Using machines less frequently on the field means less fuel

use and, consequently, lower CO<sub>2</sub> emissions. According to the English agricultural economists Graham Brookes and Peter Barfoot, 27 liters less fuel is needed per growing season to process a hectare in a no-till system, in comparison with conventional tillage farming.<sup>3</sup> Using these figures, Brookes and Barfoot worked out that in 2013, no-till farming as a result of herbicide-tolerant cultivation delivered a saving of 785 million liters of fuel. Considering that using 1 liter of fuel produces emissions of 2.7 kg of  $CO_2$ , 2 million metric tons less  $CO_2$  is emitted as a result of this fuel saving.<sup>3</sup> To make these amounts more tangible, Brookes and Barfoot calculated that in 2013, the effect of this was comparable to taking 931,000 cars off the road for a whole year. If we extrapolate that effect to the time since the introduction of herbicide-tolerant GM crops in 1996, the ensuing no-till farming means 16.8 million metric tons less in CO<sub>2</sub> emissions as a result of the 6.3-billion-liter reduction in fuel use. This is the equivalent of taking almost 7.5 million cars off the road for a year.<sup>3</sup>

Crops that are tolerant to glyphosate, the herbicide to which most herbicide-tolerant plants are resistant, appear to offer an additional environmental advantage. After all, in comparison with other herbicides, glyphosate scores

No-till soybeans thrive in wheat crops residue. This form of no-till farming reduces soil erosion and helps retain moisture for the new crop. ©Tim McCabe. Courtesy of USDA National Resources Conservation Service.



significantly better in terms of environmental impact.<sup>82</sup> If this is taken into account, since the introduction of glyphosate-tolerant cultivation, there has been a noticeable fall in environmental impact. Worldwide, this amounts to a reduction of 14.5% in environmental impact for soy, 13.5% for maize, and up to almost 28% for herbicide-tolerant rapeseed cultivation.<sup>3</sup>

### Glyphosate-tolerant crops: enough is enough

Although herbicide-tolerant cultivation has been more ecological over the last sixteen years than conventional cultivation, the guestion remains as to what the impact is when certain crops are cultivated over a very large area. In that respect, it is primarily the herbicide-tolerant soy cultivation that appears to be the victim of its own success. Driven by the low production costs of genetically modified soy cultivation and the increasing demand for soy products, soy farmers in Argentina and Brazil started producing on a greater scale.<sup>83,84</sup> Mathematical models estimate that in Argentina, more than 8.6 million extra hectares were planted because of the availability of herbicide-tolerant soy.<sup>85</sup> As GM soy cultivation is more financially appealing because of the ever-increasing demand for soy and the higher rainfall in the dry forests of Chaco, major deforestation has also taken place in South America.<sup>86,87</sup> But the danger of deforestation is no reason to prohibit herbicide-tolerant cultivation. It is, however, a reason to protect the forests in question.

The gigantic area used for cultivation of soy also raises concerns as to the negative effects of monoculture. In addition to using up certain



nutrients in the soil,<sup>88</sup> large-scale herbicidetolerant cultivation has the additional effect that very large areas are covered with the same herbicide. In the case of glyphosate-tolerant crops, this is glyphosate. As a result of the actions of environmental groups, this herbicide has come under mounting pressure over the last few years. In certain guantities, all products, including herbicides such as glyphosate, have a harmful effect on the environment. Some pesticides have even been taken off the market because their impact was deemed too great. A well-known example of this is DDT. Strict risk assessments and continuous monitoring are therefore of great importance in order to keep the impact of plant protection products as low as possible. However, in contrast to DDT, the negative image surrounding glyphosate is not confirmed by scientific data. In comparison with most other herbicides, glyphosate even has a smaller impact on the environment.<sup>89</sup> Despite

this, widespread use of glyphosate through the cultivation of glyphosate-tolerant crops is not a good thing. However, when herbicide-tolerant crops are cultivated in a well-thought-out way and according to good agricultural practice, they can indirectly contribute, through their support for no-till farming, to a significant fall in the impact of agriculture on the environment.

### Resistance to herbicides

Plant protection products must always be used with care. Products should, for example, be alternated. If this agricultural logic is not followed, there is a chance that insects, fungi, and weeds adapt and build up resistance to the product. Because the cultivation of herbicide-tolerant crops goes hand in hand with the use of a certain herbicide, it is very important to use other weedkillers (mechanic or chemical) over the years. If this does not happen, there is a substantial risk of weeds becoming tolerant to the herbicide. However, these are not "super-



weeds" that cannot be combated with any product. They are weeds that can no longer be killed by the herbicide to which they have developed resistance. As a result, after treatment, the weeds remain in the field and the efficiency of the cultivation of the herbicide-tolerant crop is reduced. From an agricultural point of view, this is a significant disadvantage. Farmers therefore have a vested interest in following the code of good agricultural practice as strictly as possible. This debate bears a strong resemblance to the debate surrounding antibiotics. It is not antibiotics that have caused the emergence of resistant hospital bacteria but rather the careless and reckless use thereof.

All weeds have the ability to develop tolerance to any herbicide. Long before the development of the first GM plant, tolerance to certain herbicides was already identified in the field. Glyphosate-tolerant weeds (Conyza bonariensis and Conyza sumatrensis) have been identified in Greece, a country that prohibits all cultivation of GM crops and where glyphosate-tolerant crops have never been cultivated.<sup>86</sup> The same is true in France, where glyphosate-tolerant rye grass (Lolium rigidum) has been identified.<sup>86</sup> In other words, not all herbicide-tolerant weeds can be attributed to the cultivation of herbicide-tolerant crops, but widespread use of glyphosate in the US has paved the way for localized resistance and accelerated its emergence. In North and South America, the introduction of herbicide-tolerant crops was a great success and everyone was so enamored by this simple method of killing weeds that almost every farmer began to cultivate herbicide-tolerant crops. The choice of the type of herbicide tolerance was limited and it was primarily glyphosate-tolerant crops that were brought onto the

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market. In Argentina, it took only eight years for almost every farmer to have swapped their conventional soy for glyphosate-tolerant soy.<sup>88</sup> In 2012, 93% of all soy in the United States came from herbicide-tolerant soy.<sup>90</sup> It is not only mass use that generates a risk of resistant weeds developing; the planting calendars used are particularly crucial. In certain areas in North and South America, where it is possible to have two soy harvests one after the other within a year, it was often two glyphosate-tolerant soy crops that were chosen year after year. In other places, glyphosate-tolerant soy was alternated with glyphosate-tolerant maize. Both of these strategies fly in the face of good sense. By relying too heavily on glyphosate as the only weedkiller and not adhering to essential cultivation principles, gigantic areas of land are treated with the same herbicide year after year. What happened was therefore only to be expected. Less than 10 years after the introduction of glyphosate-tolerant crops, the first glyphosate-tolerant weeds appeared, in both Argentina and the United States.<sup>84</sup> This led in some regions to more glyphosate being used than originally necessary or, in some cases, to farmers going back to the old, more harmful herbicides. The environmental advantage is therefore decreasing as a result of the shift in herbicide use. As a result, the environmental advantage of glyphosatetolerant soy cultivation in Argentina dropped from 21% in 2004 to only 1% in 2013.7,91

However, things can also be done differently. Herbicide-tolerant rapeseed is cultivated in Canada and alternated with wheat and rye, as a result of which it only grows on the same field every four years.<sup>92</sup> Moreover, farmers are able to use two different herbicide tolerance traits. They alternate glyphosate- and glufosinate-



tolerant GM rapeseed with each other. Glyphosate and glufosinate are two different herbicides that work in completely different ways.<sup>84</sup> In other words, a weed that is resistant to glyphosate will be tackled with glufosinate the following year. In 2010, 6.5 million hectares of rapeseed was cultivated in Canada, 47% of which was glyphosate-tolerant (GM), 46% glufosinate-tolerant (GM), 6% imazamox-/imazapyr-tolerant (non-GM) and 1% conventional rapeseed.<sup>89,93</sup> By alternating crops and type of herbicide tolerance, Canada has seen no abnormal increase, since the start of herbicide-tolerant GM cultivation, in the emergence of herbicide-tolerant weeds.<sup>86</sup> When herbicide-tolerant crops are worked with in the right way, the likelihood of obtaining tolerant weeds does not increase. The same story can be seen in Australia. Prior to the introduction of herbicide-tolerant GM crops, Australia was already dealing with weeds that had become resistant to certain herbicides.<sup>94</sup>

However, the Australians had already learned from previous agricultural errors and when herbicide-tolerant GM crops became available, Australian farmers immediately put them to good use in a careful way, and the country has seen no increase in tolerant weeds either.

Nevertheless, farmers alone cannot be blamed for the historic overuse of glyphosate, due to a lack of insight into weed control. Governments, producers of herbicide-tolerant applications, and agricultural organizations should have monitored, documented, and—where necessary—intervened in the use of herbicides and the cultivation situation. Plant biotechnology can achieve a great deal, but introducing a GM crop cannot be a reason for the basic principles of good agricultural practice being thrown overboard.

### Resistance to Bt

Just as weeds can become resistant to herbicides, insects can also adapt to plant protection products or even to cultivation practices that keep them under control. The corn rootworm (*Diabrotica virgifera*) causes great damage to maize in the United States. The larvae feed on the roots of maize plants, causing damage and loss of yield, while the beetles that grow from the larvae feed on the maize leaves and cobs. In the United States, the corn rootworm is kept



Convential maize (left) damaged by maize borers, while Bt maize (right) is resistant against these insects.

under control through annual crop rotation between maize and soy. Soy produces cysteine protease inhibitors (see page 15), which means that the larvae cannot survive on the soy roots and the risk of infection falls. However, over the years, larvae have adapted to soy, meaning that crop rotation no longer works and the rootworm can no longer be kept under control. The underlying reason for the resistance caused appears to be a change in the intestinal bacteria of the insects.<sup>95</sup>

To prevent resistance in organisms such as weeds, insects, and fungi, there must be integrated crop protection, which involves using several products or techniques simultaneously against a particular pest. In the past, this happened too little. As a result, the corn rootworm could adapt to the insect-resistant Bt crops and consequently undermine the effectiveness of maize/soy rotation. It quickly became clear from laboratory experiments that insects can quite quickly adapt to Bt proteins. It was already proven in the late 1990s that the European stalk borer could become resistant to a specific Bt crop after eight generations of selection.<sup>96</sup> Given the success of Bt cotton and Bt maize, insects such as the cotton bollworm eventually becoming insensitive to Bt proteins would be a farmer's nightmare. Bt plants would then become less efficient at combating the damage from insects, which would take the insect problem back to square one. Governments and producers therefore encouraged farmers to use certain cultivation measures to combat the emergence of Bt-resistant insects (see box "Overcoming Bt resistance through good agricultural practice"). In some cases, these precautions were not heeded enough.97 Five types of insect with resistance to a

Bt protein have now been identified in the field, resulting in less effective protection against these insects for the associated Bt crops (both maize and cotton) in certain areas.<sup>97</sup>

Farmers can combat Bt resistance with good agricultural practices. The producer also bears responsibility in this regard. If we want to cultivate sustainably, crops with single defense mechanisms should be avoided. Governments can also stimulate good agricultural practice and discourage one-sided cultivation. The most efficient way to repress resistance is to let crops produce different defense mechanisms at the same time. Maize and cotton with two or more different Bt proteins working against the same insect have a more sustainable defense system. If the probability of resistance to a Bt protein is 1 in 100,000, the probability of resistance to two different Bt proteins developing at the same time instantly becomes 1 in 10 billion. This also applies to conventionally cultivated crops. With the latter, however, we do not know in most cases what the resistance is based on and whether there are one or more defense mechanisms responsible for it.

To conclude this section, it should be highlighted that not all insects that are resistant to one or more Bt proteins have developed this resistance as a result of the cultivation of Bt crops. Even before the commercial cultivation of Bt crops began, insect resistance to certain Bt proteins was identified in the field.<sup>98</sup> This resistance was attributable to the use of sprays based on Bt.

### OVERCOMING BT RESISTANCE THROUGH GOOD AGRICULTURAL PRACTICE

A number of issues should be understood in order to prevent resistance to Bt proteins. The first is the dosage rule. This rule is the crux of controlling Bt-resistant insects. The rule states that Bt plants should produce a sufficiently high dose of Bt proteins to kill the majority (>99%) of the target insects. If the dose is too low and certain insects can survive a Bt meal, they can gradually adapt to it and produce less susceptible descendants. In such a case, a Bt field would be full of Bt-resistant insects in no time at all.

The second crucial measure is stacking defense mechanisms. The chance that a spontaneous mutation in the DNA of an insect leads to resistance against a Bt protein is very remote. This remote chance of resistance decreases even more dramatically with the production of two Bt proteins in a plant that work against the same insect but with a different mode of action. After all, a cotton bollworm that has become resistant to one toxin will still be sensitive to the other. As a result, the insect is always killed off and the resistance acquired is eliminated from the population. In Australia, India, and the United States, people adopted this solution massively. Around 90% of Indian Bt cotton plants already contain two defense mechanisms; in Australia and the United States, this figure is 100%.<sup>7,99</sup> As a result, the chance of breaking down the plants' resistance is smaller. Despite this, multiple defense mechanisms should not be considered a miracle cure. Measures still need to be taken. For example, crops with a single defense mechanism should not be cultivated with crops with multiple defense mechanisms. After all, insects would gradually have time to adapt and the multiple defense would be broken down more quickly. Between 2004 and 2010, GM cotton that produced one or two different Bt proteins was cultivated in the US. Nowadays the frequency of resistance to both Bt proteins is more than 50% in some insects. In Australia, the frequency of resistance now stands at less than 1%.<sup>99</sup>

Another important cultivation strategy is the "refuge" rule. In the USA, Australia, and other countries, government legislation ensures that farmers cultivating Bt cotton or Bt maize must also plant refuges alongside their *Bt fields consisting of strips of non-Bt cotton or non-Bt maize (crop refuge area). The idea is that if an insect* becomes resistant, it will in the first instance mate with a Bt-sensitive insect present in the crop refuge area. If the mutation delivers a genetically recessive trait, descendants of such a cross-breed become Bt-sensitive. If these eggs are laid on a Bt plant, the larvae are killed off after a Bt meal and the resistance is eliminated from the field. A comparable scenario is hoped for with the release of sterile adult insects.<sup>100</sup> These insects are bred in the laboratory, after which they are irradiated to prevent them from producing any descendants. The sterile insects are then released into the field in large quantities. Because of the excess of sterile insects, any insects that have become resistant in the Bt field mate in the first instance with the released sterile insects. In such a case, there are no more descendants, meaning that the resistance dies a quiet death. Between 2006 and 2009, around 2 billion sterile Pectinophora gossypiella moths ("pink bollworms") were released in the cotton fields of the state of Arizona (or 25,000 sterile moths per hectare per year). This pest-control exercise was successful: in 2009, only 2 larvae were counted in 16,600 cotton balls compared with 2,550 larvae in 2005. The level of infection fell from 15.3% to 0.012%, amounting to a decrease of 99.9%. The same decrease was observed in the number of moths that fell weekly into insect traps.<sup>101</sup>



### Indirect additional plant protection

A large-scale study in which measurements were taken over 20 years in 36 different locations in China studied the presence of ladybugs, lacewings, and spiders in Bt cotton fields.<sup>102</sup> The data collated showed that the number of these useful insects and spiders had increased: an increase that the scientists fully attributed to the decreased use of broad-spectrum insecticides. Because the cotton bollworm no longer needs to be controlled by spraying with broad-spectrum insecticides, other insects in the cotton field are no longer killed. Ladybugs, lacewings, and spiders are often considered predators because they are natural foes of pest insects. In other words, these predators can control pests naturally. This is, for example, the reason why ladybugs and parasitoid wasps are used in organic greenhouse vegetable cultivation. The Chinese researchers noticed that these useful predators spread to surrounding maize, groundnut, and soy fields. In these neighboring non-Bt crops, they also controlled pest insects, reducing the need for spraying even in the non-Bt fields.<sup>102</sup> In this case, the surrounding non-GM fields benefit not only from the overall reduced disease burden but also from the greater number of useful insects. In other words, Bt technology can offer vital help in moving toward agriculture with less use of chemical pesticides and a greater focus on biological pest control.

The positive effects reported in the Chinese study cannot always be directly extrapolated to other studies. The effect of Bt crops on non-target organisms appears to depend not only on the crop but also on the insect. In 2007, the journal Science came out with a meta-analysis that used 42 field experiments to examine a wide range of non-target insects.<sup>103</sup> The analysis brought to light the finding that certain non-target organisms were present in lesser quantities in Bt fields than in insecticide-free control fields. However, their number in Bt fields was far greater than in fields treated in the conventional way with insecticides. A study published later, which included 21 additional field tests, came to the same conclusion.<sup>36</sup>

The reduced number of insects in Bt fields in comparison with untreated non-Bt fields may have two causes. On one hand, this may be a toxic effect through non-target organisms being directly influenced by the Bt plants or indirectly through eating prey insects that fed on the Bt plants. On the other hand, it may be an ecological effect. Certain insects (predators) have a lesser presence because they have to look for their prey elsewhere, with fewer Bt-sensitive prey insects in the field. Data from the latest big meta-analysis study leans toward an ecological effect.<sup>36</sup> After all, laboratory studies demonstrated that certain predators with less of a presence in a Bt field were themselves not sensitive to Bt proteins. They were, on the other hand, almost exclusively dependent on corn borer caterpillars. Because Bt crops were developed to combat corn borers, it follows that there will also be fewer predators in the Bt fields. It must, however, be noted that when corn borers are controlled in another way (e.g. by spraying Bt or by using other means), these

specific predators will also be less present. The most important conclusion from both meta-analysis studies was that when Bt fields are compared with insecticide-treated non-Bt fields, many more useful insects are found in the Bt fields.<sup>36,103</sup>

### Secondary pest problems

The reduced use of insecticides in the cultivation of insect-resistant Bt crops can, in certain circumstances, have an indirect negative effect. Following a 10-year field study in the province of Heibei in northern China, Chinese researchers noted a steady increase in the population of leaf bugs in Bt cotton fields.<sup>104</sup> These bugs are sensitive to broad-spectrum insecticides used against the bollworm in conventional cotton cultivation. However, because of reduced insecticide use in Bt cotton, the bugs are less under control and they can cause secondary pest problems. In the USA, there is a similar problem with the Western bean cutworm in maize. This insect is only partly sensitive to the Bt maize currently being cultivated. As a result of the significant decrease in the use of broad-spectrum insecticides and the reduced presence of insects that are controlled by Bt maize, the insect is much more prevalent than prior to the introduction of Bt maize.<sup>105</sup>

Although opponents of GM technology view this as proof that Bt technology is not a sustainable solution, these results in fact show the positive aspects of Bt crops at play. The Bt proteins in GM crops are so specific in their action against target organisms that insects such as leaf bugs and aphids are not killed when they feed on the crops. Moreover, these non-target organisms can only grow into a plague if a reduced quantity of broad-spectrum insecticides is actually used in Bt fields. Secondary pest problems can be prevented by developing additional genetic resistance and introducing it to Bt crops, or by researching other crop protection methods (biological pest control or insecticides with a more specific action). A more biological form of pest control is possible precisely because a smaller quantity of plant protection products is used. In the short term there is also the chance that farmers will go back to the original insecticides because this is the simplest and cheapest way. Bt technology is not a miracle cure that solves all crop problems in one fell swoop. It is, however, an important tool for a step-by-step evolution toward sustainable farming with as little impact as possible on the environment.





# The European environmental risk assessment for GMO cultivation

A GM crop may only be cultivated commercially in the European Union after it has been subjected to an environmental risk assessment. The European legislation in question is very strict and imposes in-depth assessment. A company that wishes to place a GM crop on the market must submit a comprehensive file with the results of the assessments. The file is then rigorously inspected by the European Food Safety Authority (EFSA) or one of the EU Member States. The final decision for granting the requested approval for the GM crop lies in the hands of the European Commission and the Member States. Here we describe, in technical detail, what sort of assessments this file must include. For the purpose of full clarity, it must be noted that the stringent regularization process that GM plants have to go through with official food safety bodies does not apply to crops that have received traits through conventional and mutation breeding. Scientifically speaking, it would be more logical to evaluate new crops on the basis of their new traits, not on the basis of the breeding technique with which they were genetically modified.

### A comparative study with the non-GM variant

In 2010, the GMO panel of the EFSA imposed guidelines that a cultivation application must comply with.<sup>106</sup> The application file for the cultivation of GM crops in Europe has to include a food safety study (at least for food and feed) and an environmental study. The experiments conducted and evaluated during these food safety studies are explained in full detail in the background report "Food safety of genetically modified crops".<sup>1</sup> The data provided by the environmental study must enable the EU Member States and EFSA to assess whether the introduction of a specific GM plant into the environment could have negative effects on the environment, human health, or animal health. This looks into both the intended and unintended consequences of genetic modification. The intended effects are those for which the GM plant was developed. The intended effect of an insect-resistant plant, for example, is to eliminate damage from specific pests. Unintended effects can be, for example, a consequence of differences between GM and non-GM plants, such as the place in the DNA of the plant where the genes are built in. To be able to trace the unintended effects, a comparative study is carried out between the GM plant and the non-GM variant.

To obtain an approval for cultivation, an application file must also include specific assessments with regard to the interaction of the GM plant with the environment. All of these assessments first ascertain which aspects of the GM plant could produce a negative consequence for the environment. The actual risk is then determined by taking into account the environment's exposure to the possible negative aspects of the GM plant.

### Invasiveness and vertical gene transfer to other plants.

The introduction of plants or animals into an area in which they are not yet present must always be closely monitored. After all, there are sufficient examples from the past where the intended or unintended release of certain animals or plants had a dramatic impact on the fauna and flora of a particular area (see page 28).

As regards invasiveness, EFSA considers the risk on two levels. On one hand, there is the possibility that the GM plant, or wild relatives of the GM plant that have received the GM trait through cross-breeding (see page 22), could proliferate in the field. This would mean, for example, more intensive weed control strategies being applied, which in turn could have a more pronounced effect on the environment. On the other hand, it considers the effect that the GM trait could have outside the field. If the plant that has received the GM trait proliferates, could this put pressure on other plants, and could this have an effect on biodiversity? This can also have a significant effect on certain types of animal and/ or insects that use the plant for food, protection, or reproduction.

In the assessment of a possible change in invasiveness, what is studied first is whether the general biological traits, such as the seeds' capacity to germinate, are changed in the GM crop. If this is not the case, the risk of invasiveness in both cases lies in the extent of the GM trait's effect on whether the plant proliferates and the chance of the GM trait being passed on through cross-breeding to wild plant varieties (vertical gene transfer). The evaluation of this risk is therefore almost fully dependent on the GM trait and the plant type used (Table 1). The data used for this risk assessment is obtained from scientific literature or supplied by the applicant of the approval procedure.<sup>104</sup>

Trait	Сгор
Seed germination or germination capacity	Reproduction biology
Dormancy period or the extent to which seeds can lay dor- mant for several years without losing germination capacity	Propagation of pollen, seeds, tubers, bulbs, etc. in the environment
Speed of growth of seedling	Longevity of pollen, seeds, tubers, bulbs, etc. over time
Seed production	Availability of wild variants
Resistance to abiotic stress (cold, drought, etc.)	Adaptation to growth conditions
Resistance to biotic stress (fungi, insects, etc.)	

Table 1. Examples of factors that stimulate invasiveness and unintended spread of crop traits and are taken into account during the risk assessment carried out by EFSA.

### Horizontal gene transfer between plants and microorganisms

As a result of crop cultivation, plant DNA (and therefore also DNA from a GM plant) can end up in the environment: leaves fall on the ground and are digested by soil organisms, root systems and stubble from maize, for example, are left behind in the field, animals and insects eat the living or dead plant material, etc. In its GM risk assessment, EFSA takes into account the possibility that the DNA of a plant can be transferred to microorganisms such as bacteria and fungi, which is called horizontal gene transfer (see page 27). During the risk assessment, the likelihood of the added DNA fragment being transferred to non-GM microorganisms is studied. The chance of a horizontal gene transfer of this kind is incredibly small. However, when there is a great similarity between the DNA fragment added to the GM plant and DNA fragments of a certain microorganism, the chance of horizontal gene transfer increases. The same applies if there is a potential selective advantage for the microorganism with the incorporation of an extra piece of DNA or if the GM plant material does not decompose and stays in the environment for a long time. All of these factors are taken into account.

The potential consequence of a horizontal gene transfer of this kind for people, animals, and the environment is also evaluated. Particular attention is placed on the use of antibiotic resistance genes. For more information on this subject, see the background report "Food safety of genetically modified crops".<sup>1</sup>

### Interaction between GM plants and target organisms

Pest- or disease-resistant crops have strategies that enable them to defend themselves against certain insects, bacteria, fungi, or viruses. This can be achieved with the production of certain proteins such as the Bt proteins from *Bacillus* thuringiensis (see Box "Current GM applications" and the background report "Bt cotton for India"<sup>34</sup>), which are harmful to insects. Target organisms are those organisms against which a strategy is developed. All other organisms are considered non-target organisms (see following paragraph). Naturally, the ideal scenario is that the target organisms be repelled as efficiently as possible without also having a negative effect on the non-target organisms. EFSA (or one of the EU Member States) conducts a risk assessment to determine the extent to which this can be expected for the specific GM crop on which they have to formulate a scientific opinion. Some of the aspects considered are: the mechanism of the introduced protein that defends the plant, the life cycle of the target organism, the different types of host used by the target organism, and the geographical spread and mobility of the target organism.

In addition, the applicant has to submit cultivation strategies to EFSA as regards preventing circumvention of the plant's defense for as long as possible. After all, insects, bacteria, and fungi adapt to less favorable circumstances such as the cultivation of GM crops with a defense mechanism or the use of plant protection products. If plants with a defense mechanism against an insect or fungi are used, over time,



through selection, insect and fungi variants will appear that can circumvent the defense mechanism in the plant. To combat this as much as possible, the applicant of the file has to show that the target organisms are tackled efficiently. For example, the production of the defense molecules has to be great enough (depending on the absorption of plant material by the attacker) that the attacker is unable to survive (see box "Overcoming Bt resistance through good agricultural practice"). It is also essential to determine whether there are already methods available in the natural populations of target organisms to break down the plant's defense. The evaluation must also take into account the potential risk that target organisms can break down the defense. Resistance management is not required, however, for the introduction of crops that obtain defense mechanisms through conventional breeding, such as the potato varieties Toluca, Bionica, and Carolus (see background report "A blight resistant potato for Europe"4).

#### Interaction between GM plants and non-target organisms

An important aspect of the risk assessment prior to approval is examining whether the GM plant and the cultivation thereof can be a direct or indirect danger to non-target organisms. Non-target organisms, as explained above, are organisms that have no disadvantageous effect on the growth and/or development of the crop, so do not need to be combated. Non-target organisms can, however, be unintentionally influenced both by the newly introduced traits of a crop and by specific cultivation measures linked with the GM plant. If this is the case, the cultivation of the GM plant can affect the biodiversity of the ecosystem.

Because there are a great deal of non-target organisms in every ecosystem, it is impossible to test them all for direct and/or indirect sensitivity. This is why non-target organisms are divided into five categories and a number of samples are selected that are representative for each group. These different categories are: plant-eaters, natural enemies, pollinators, composters, and plant symbionts (varieties that have a symbiotic or mutually beneficial relationship with the crop). For these organisms, it is necessary to investigate the extent to which they can come into contact with the specific GM crop, with which parts of the plant, in what way, and during what stage of their life cycle. It is important to determine to what extent the non-target organisms are sensitive to the crop trait of the GM plants or to a change in agricultural practice, how abundant they are, how they interact with target organisms, and how important they are to the ecosystem.<sup>106</sup>

The aforementioned assessments should not only be conducted for bitrophic interactions (plant/non-target organism) but also for interactions involving more than two different organisms (multitrophic interactions). An organism (e.g. earthworm) that comes into direct contact with the GM crop (e.g. a leaf that has fallen off an insect-resistant plant) may perhaps encounter no problems with the crop trait, but when this organism functions as prey for another organism (e.g. a bird) it cannot simply be ruled out that the latter will encounter no negative effects over time. Some harmful crop traits can, after all, accumulate in the food chain. All data necessary for the risk assessment of non-target organisms are obtained from laboratory tests or during field tests.

All of these aforementioned assessments make it clear that the result of the risk assessment relies heavily on the crop, the incorporated trait, and the ecosystem.

### Impact of specific cultivation, management, and harvest techniques

The introduction of a GM plant in the field may go hand in hand with specific changes in cultivation, management, and harvest techniques. The effect of these changes on the environment and biodiversity must also be investigated before a GM crop receives approval for cultivation.

The most obvious examples of this are herbicide-tolerant crops. The cultivation of these crops is inextricably linked to the use of one or more specific herbicides. In most cases, this will entail a shift in herbicide use, meaning that the herbicides the crop is tolerant to will be used more, while the use of other herbicides will decrease. The effect of this must therefore be investigated, including the biodiversity in and around the field and what the direct and indirect consequences are. With flexible and efficient weed control, in combination with the cultivation of certain herbicide-tolerant crops, farmers can more easily switch to no-till farming (see page 31 and background report "Herbicide resistant soybean in Argentina"84). This form of agriculture is recommended in areas with erosion-sensitive soils to improve the soil structure but has a significant effect on living organisms in and on the soil.

### Effects on biochemical processes

Biochemical processes include the movement, storage, and conversion of energy, water, carbon, nitrogen, and other elements in the ecosystem. Examples of this include uptake of CO<sub>2</sub> from the atmosphere by plants, decomposition of plant material, formation of organic substances in the soil, evaporation of water from the soil, conversion of nitrogen, etc. Biogeochemical processes are essential to build up soil fertility but they also contribute to the loss of certain components in the soil, e.g. the release of greenhouse gases (CO<sub>2</sub>, methane, and nitrous oxide), which has a negative impact on the environment. Applicants for an approval for GM cultivation must supply data for the purposes of ascertaining whether the GM plant and the associated cultivation method could potentially have a negative effect on the biogeochemical processes in comparison with the current production methods and the non-GM variant.<sup>106</sup>

### Effects on humans and animals

The approval procedure for the cultivation of the GM crop naturally focuses on the potential effect of the GM crop and the associated cultivation method on the environment, but additionally includes a risk assessment to explore the potential effects on the health of humans and animals.<sup>106</sup> After all, farmers, animals, and to a lesser extent passers-by do come into contact with agricultural crops and/or are exposed to pollen and substances coming



from these plants. It is therefore important to be able to exclude the possibility of an increased risk being incurred through cultivation of a GM crop in comparison with a non-GM crop as regards human and animal health. This is why these GM crops or parts thereof, such as pollen, need to be evaluated as regards their potential allergenicity and contact toxicity. Moreover, accidental intake, even of crops not intended for consumption, such as cut flowers, cannot be ruled out. Accidental intake is also taken into account in the risk assessment. In practice, it is therefore clear that a GM crop for which there is an application for an approval for cultivation must undergo both a risk assessment as regards potential effects on the environment and biodiversity and a risk assessment for potential health effects on humans and animals.

#### Post-market monitoring or follow-up of commercial cultivation

When EFSA delivers a positive opinion to the European Commission, the European policymakers can approve a GM crop for cultivation. In addition to the risk assessment conducted prior to the approval for cultivation, the approved product must continue to be monitored in the field, even after its cultivation is approved. This is primarily done to ascertain whether the crop is used as it was intended, to check the expected effects of the crop, and/or to identify whether there are any unexpected side effects. For an herbicide-tolerant and insect-resistant crop, for example, it is necessary to monitor the potential development of resistant weeds and insects.

## 6 Conclusion

There are an enormous number of variables that determine impact on the environment. Alongside the method of cultivation, it is primarily the traits of a specific crop (yield capacity, disease resistance,...) that determine environmental burden. However, these two parameters (method of cultivation and crop traits) are entirely independent of the technology used to breed the crop. All crops bred, whether obtained through "traditional" breeding techniques or through GM technology, can have an impact on the environment.

From a regulatory standpoint, however, a great distinction is made between the two technologies. Whilst the products of traditional and mutation breeding are free to be placed on the European market, strict risk assessment is required in order to investigate the potential effects of GM crops on the environment. In this context, GM crops are compared with non-GM crops cultivated through conventional agricultural practices.

Just like crops with specific traits, insect-resistant, drought-tolerant, and virus-resistant crops all have direct and indirect effects on the environment. These effects can be either positive or negative but in most cases they will have both positive and negative elements. It is therefore crucial to conduct the risk assessment on a case-by-case basis and to evaluate the environmental impact for the approval for cultivation on the basis of a cost/benefit analysis. In other words: in comparison with the advantages of a certain crop, what is the acceptable and unacceptable impact on the environment?

The scope of environmental risk assessments and strict regulations ensure that the only GM crops brought onto the market are those that do not have a greater negative impact on the environment than their non-GM counterparts.



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#### VIB

Rijvisschestraat 120 9052 Ghent Belgium Tel. +32 9 244 66 11 Fax +32 9 244 66 10 info@vib.be www.vib.be

R.E. Jo Bury, Rijvisschestraat 120, 9052 Ghent, Belgium - D/2016/12/267/6

