

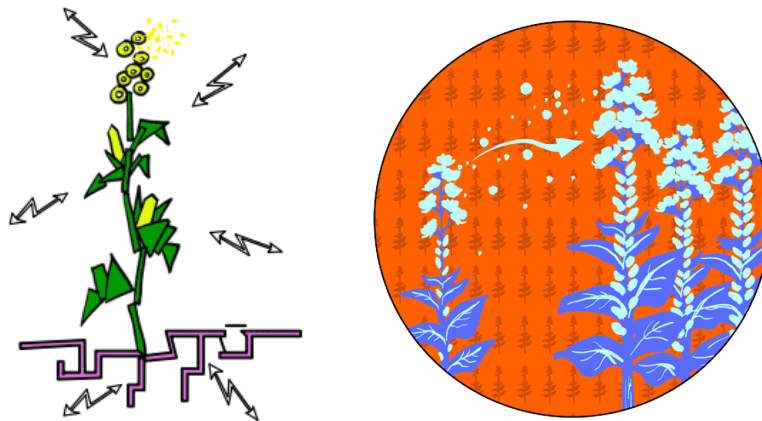
RAGES

RISK ASSESSMENT OF GENETICALLY ENGINEERED ORGANISMS IN THE EU AND SWITZERLAND

Environmental risk assessment of genetically engineered crops that can persist and spontaneously propagate in the environment

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January 2020



Funded by:

**STIFTUNG
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SCHWEIZ**

Editorial:

Bauer Pankus A., Miyazaki J., Then C., (2020) Environmental risk assessment of genetically engineered crops that can persist and spontaneously propagate in the environment. Report of the results from the RAGES project 2016-2019, www.testbiotech.org/projekt_rages

Published by ENSSER (<https://ensser.org>), GeneWatch UK (www.genewatch.org) and Testbiotech (www.testbiotech.org).

Figures: Testbiotech, Timo Zett, Claudia Radig-Willy

Funded by Stiftung Mercator Schweiz

Content

Summary.....	4
1. Introduction.....	6
2. Enhanced fitness and risk assessment of genetically engineered plants.....	6
2.1. Enhanced fitness is intended by the trait.....	7
2.2 Enhanced fitness unintentionally associated with the trait.....	7
2.3 Unexpected changes in fitness emerging from genomic effects.....	8
2.4 Changes in fitness can be triggered by genome x environmental interactions.....	11
3. Other biological characteristics and environmental interactions of relevance.....	13
4. Problems assessing long term and next generation effects.....	14
5. Regulatory options to take into account spatio-temporal complexity.....	19
6. A case study: Risk assessment of GE oilseed rape.....	23
7. Conclusions.....	25
References.....	27

Summary

If genetically engineered (GE) organisms can persist and propagate in the environment and produce viable offspring, new challenges for risk assessment arise. In this review it is shown next generation effects can be influenced substantially by interactions with heterogeneous genetic backgrounds. Furthermore, unexpected effects can be triggered in interaction with environmental conditions. This observation is especially relevant for the assessment of long term impacts under changing environmental conditions such as caused by climate change. Therefore, risk assessment of genetically engineered plants that can persist and propagate in the environment cannot be reduced to the specific traits and characteristics that are known at the stage of application, but also has to take into account effects that can emerge after some generations, in other genetic backgrounds or under stress conditions.

However, in regard to environmental risk assessment (ERA) as currently performed by the European Food Safety Authority (EFSA), the safety of the next generation resulting from spontaneous propagation is hardly considered. Furthermore, the only potential hazards as identified by EFSA are exacerbating weed problems, displacement or even extinction of native plant species. However, these potential hazards are not the only risks that can arise from the persistence and self propagation of GE crops. Potential hazards concern the plant's interactions and biological signaling pathways within the food web, with soil organisms or insects such as pollinators and other organisms. These pathways and networks can be disturbed or disrupted, for example, by changes in the composition of volatile compounds or biochemical pathways and changes in nutritional quality.

In general, risk assessment of GE organisms which can persist and spontaneously propagate in the environment (within or beyond their production systems) has to deal with a spatio-temporal dimension that is far more complex compared to GE plants that are only grown for one season. Depending on the specific spatio-temporal dimensions, the environmental risk assessment poses problems for both risk assessment and risk management, because of a high level of uncertainty.

To deal with these problems, we recommend establishing 'cut off criteria' in risk assessment that take into account the factual limits of knowledge. It is proposed to introduce these 'cut-off criteria', based on a specific step of 'spatio-temporal controllability' within risk assessment. This new step combines three criteria:

- (1) the natural biology of the target organisms,
- (2) their naturally occurring interactions with the environment (biotic and abiotic)
- (3) the intended biological characteristics of the genetically engineered organisms.

The combination of these three criteria in one specific, additional step in risk assessment has the advantage of them already being used to some extent in current EFSA risk assessment; many of the details to assess these criteria are very well known. This concept can be used to delineate some of the boundaries between what is known and those unknowns considered to be crucial. Consequently, this additional step in risk assessment will foster the robustness of risk assessment and can substantially benefit the reliability of decision making within approval processes.

It is suggested that, in cases where it is known that GE organisms can escape 'spatio-temporal controllability' because they can propagate within natural populations with no effective control of spread or persistence, then the authorisation process cannot proceed and the release of the GE organism cannot be allowed. The reason for determining the approval process under these conditions is lack of conclusiveness of the risk assessment.

The criteria as suggested should not only be applied to applications for commercial cultivation but also to imports that are likely to cause spillage of viable kernels of relevant events / species. In general, the release of genetically engineered plants should not be allowed if their persistence in the environment cannot be controlled in the spatio-temporal dimension.

1. Introduction

According to European Food Safety Authority (EFSA), there are specific hazards which have to be assessed in risk assessment of GE plants in regard to “Persistence and invasiveness including plant-to-plant gene flow” (EFSA 2010, page 40). All of them involve a change of fitness:

“The potential adverse effects are of two main types. First, enhanced fitness of the GM plant or of transgenic (introgressed) wild relatives within production systems may make them more persistent, exacerbating weed problems that may need to be controlled by more complex weed control strategies, which themselves might cause environmental harm. Second, enhanced fitness of transgenic feral plants, or of transgenic (introgressed) wild relatives in semi-natural or natural habitats may reduce the diversity/abundance of valued flora and fauna. For instance, native plant species may be displaced, which in turn might affect species that use those plants as food, shelter, etc. Alternatively, and depending on which plant and which transgenes are involved, gene flow to wild relatives may decrease the fitness of hybrid offspring. If rates of gene flow are high, this may cause wild relatives to decline locally, or to become extinct (e.g. swarm effect, outbreeding depression).”

This review investigates the question of how changes in the fitness of volunteer offspring and hybrids of genetically plants can be assessed. More generally, we explore whether the risk assessment as established by EFSA (2010) is sufficient to identify all relevant hazards that might emerge from the persistence and spread of genetically engineered organisms in the environment.

For this purpose, we present a review of effects that can cause a change of fitness in genetically engineered plants. Further we discuss which other biological characteristics (besides a change in fitness) are relevant for environmental risk assessment of GE plants in this context. Finally we draw some conclusions and identify specific regulatory recommendations.

2. Enhanced fitness and risk assessment of genetically engineered plants

Fitness is a term used in evolutionary biology. Within sexually reproductive populations of a species, fitness can be quantified by comparing the reproductive success of specific individuals carrying certain properties, compared to the reproductive success of those lacking these properties. If the reproductive success of the specific individuals is higher than that of the rest of the population, this effect can be called enhanced fitness (EF). In general, fitness is dependent on the environment: some individuals might be better prepared to survive under specific environmental stressors than others.

In the context of risk assessment of genetically engineered plants with enhanced fitness (EF), we propose four categories. The enhancement can be (1) intended by the trait, or (2) unintentionally

associated with the trait. Further, enhanced fitness can (3) emerge from unintended biological effects, for example after hybridisation with wild relatives. Finally (4) it might be triggered by specific environmental conditions. These four categories will be exemplified in the following.

2.1. Enhanced fitness is intended by the trait

There are several reasons for developing genetically engineered plants with enhanced fitness:

- Due to climate change, more extreme weather conditions will be common in the future. Therefore, genetic engineering is sometimes used to attempt to adapt food crops. For example, maize plants are cultivated in the US (MON87460) that are claimed to be tolerant to drought conditions.
- To attempt to increase their yield, crop plants might be manipulated to show higher efficiency in using input factors such as nutrients or sunlight.
- Another approach for increasing yield is maize MON87043 that is supposed to produce larger corn ears and more kernels.
- Another aim is to improve cultivation of plants under disadvantageous soil conditions such as high level of salinity.
- While many approaches have been discussed (Bhatnagar-Mathur et al., 2008; Cabello et al., 2014; Khan et al., 2016, Castiglioni et al. 2008), so far only a few have been realised in commercial cultivation (see Table 1).

Table 1: Some examples of genetically engineered plants with intended enhanced fitness authorized or deregulated for cultivation

Species	Trait	Developer	Comment / regulatory status
Eucalyptus	frost tolerance	ArborGen	Applied for cultivation in Brazil
Maize (Event MON87460)	drought tolerance	Monsanto	also stacked with herbicide resistance (Glyphosate) and insecticidal toxicity (Bt toxin); approved for cultivation in the US
Soybean (Event IND-ØØ41Ø-5)	drought tolerance	Verdeca	approved for cultivation in Argentina
Soybean (Event MON87712)	yield increase by modulation of the plant's metabolism in response to dark-to-light transition	Monsanto	approved for cultivation in the US
Sugarcane (Events NXI-1T, NXI-4T, NXI-6T)	drought tolerance	'Persero'	applied for cultivation in Indonesia
Maize (Event MON87043)	larger corn ears and higher yield in kernels	Monsanto	applied for import in the EU

2.2 Enhanced fitness unintentionally associated with the trait

Traits intended to enhance production by introducing herbicide resistance, insecticidal properties or tolerance to biotic stressors such as viruses can also confer enhanced fitness under specific environmental conditions.

For example, genetically engineered plants with herbicide resistance established in parts of the

environment, such as ruderal areas (waste ground) or transport routes where the complementary herbicide is being used to control weeds, have been shown to have a higher survival rate (see Londo et al., 2011a; Londo et al., 2011b). If the herbicide they are made resistant to is used frequently, such as in the case of glyphosate, this can become an issue for risk assessment.

Furthermore, if plants made tolerant to biotic stressors such as pest insects, viruses or fungal diseases, are established in the environment, the genetically engineered plants will show higher survival rates if exposed to these biotic stressors. For example, genetically engineered rice showed a higher fitness in experiments where the plants were under insect pest pressure (Zhang et al., 2012). If introgressed into wild relatives, the offspring can benefit from the trait (Lu and Yang, 2009; Letourneau and Hagen, 2012; Meier et al., 2013; Mason et al., 2011; Mason et al., 2003; Aono et al., 2006; Hjältén et al., 2012). Similar observations were made with virus resistance in squash (Laughlin et al., 2009), and radish (Snow et al., 2001).

It also has to be taken into account that there are several plants with a combination of relevant traits such as insecticidal properties and resistance to one or several herbicides. For example, genetically engineered maize sold as “SmartStax” (MON89034 x 1507 x MON89017 x DAS59122) produces six insecticidal proteins and is made resistant to two herbicides. The combined impacts, potential synergistic, additional or antagonistic effects of these traits can also influence its overall fitness.

Table 2 gives an overview of some traits that show enhanced fitness in regard to biotic stressors.

Table 2: Some examples of genetically engineered plants with enhanced tolerance to biotic stressors authorized or deregulated for cultivation

Species	Traits	Developers
Cotton	Insecticidal	Monsanto, DuPont
Egg plant	Insecticidal	Monsanto
Maize	Insecticidal	Monsanto, DuPont, Dow AgroSciences, Syngenta
Papaya	Resistance to viral infection, papaya ringspot virus (PRSV)	Cornell University, US, and others
Plum tree	Resistance to plum pox virus (PPV)	USDA
Poplar	Insecticidal	Grown in China
Potato	Fungal disease tolerance	J.R. Simplot Co.
Soybean	Insecticidal	Monsanto, DuPont

2.3 Unexpected changes in fitness emerging from genomic effects

The process of genetic engineering may result in lower fitness of the resulting plants compared to their isogenic comparator. There can be several reasons for this, one is that the metabolic pathway introduced into the plants might imply some fitness costs (for discussion about fitness costs of induced resistance in plants see, for example, Heil and Baldwin, 2002). In other cases, lower fitness is caused by unintended effects such as interruption of endogenous genes. For example, Bollinedi et al., (2017) after crossing lines of so-called “Golden Rice” with the Indian variety Swarna observed growth disturbance, since the gene constructs interfered with the plant’s own gene for producing growth hormones. Further, gene constructs were not, as intended, active solely in the kernels, but also in the leaves. This led to a substantial reduction in the content of chlorophyll that is essential for vital functions in the plants, and hence lower fitness.

The process of genetic engineering can also unintentionally enhance fitness of the relevant plants. For example, the number of pollen and seeds or responses to environmental stress conditions might be changed. There is evidence that these effects occur due to the transformation process of genetic engineering: Fang et al. (2018) showed that higher fitness does occur in genetically engineered glyphosate resistant plants in a glyphosate-free environment. According to the research from China, the enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) produced in the plants not only makes the plants resistant to glyphosate, it also interferes with plant metabolism for growth and fecundity. As a consequence, plant offspring can produce more seeds and be more resistant (tolerant) to environmental stressors such as drought and heat. The Chinese researchers stated that the observed effects are likely to be caused by increased production of the hormone auxin in the transgenic plants. This plant hormone plays a key role in growth, fecundity and adaptation to environmental stressors.

In the case of glyphosate resistant plants (Fang et al., 2018), it was shown that higher fitness was caused by the gene inserted coding for EPSPS. There are other cases, where higher fitness emerges from position effects due to the insertion site of the additional DNA. For example, in the patent application WO 2004053055, the company Monsanto claims transgenic plants emerging from the process of transformation “producing unexpected but yet desired phenotypes”. As described in the patent: “One aspect of the invention provides transgenic maize seed for maize line which exhibits enhanced yield as compared to yield for parental maize line, in another aspect the invention provides transgenic maize seed for a maize line characterized by enhanced yield under stress conditions. In another aspect the invention provides transgenic maize seed for maize lines characterized by other enhanced traits, e.g. an enhanced quality in plant morphology, plant physiology or seed component phenotype as compared to a corresponding phenotype of a parental maize line.” There are also publications showing enhanced fitness occurring from introgressing of genetically engineered plants into genetic backgrounds of other varieties or relatives (see for example in sunflowers, Snow et al., 2003; see also table 3).

In the following, we provide a tabled overview on changed fitness due to unexpected effects occurring with the process of genetic engineering, using two plant species as example, rice and oilseed rape.

Rice is chosen because of its possibility for hybridisation with wild relatives. Domesticated grasses (*Poaceae*) still have a high potential for persistence and invasiveness. Rice provides a useful example here since it has a history of double domestication (or re-domestication) with periods in between of “de-domestication”, or reversion to a wild form (Vigueira et al., 2013; Kanapeckas et al., 2016). Consequently, gene flow between wild (weedy rice) and cultivated rice forms growing in vicinity is extensive (Chen et al., 2004). The gene flow between fields and weedy rice can also be circular, reiterative and repetitive (see also Lu and Snow, 2005).

Transgenic oilseed rape is known to be established independently from cultivation in several regions of the world such as Canada, the US, Japan, Australia and Switzerland. In some countries, such as Canada and Japan it has to be assumed that there has already been some gene flow of transgenes into populations of wild relatives. Apart from commercial cultivation (such as that in Canada and the US) and experimental field trials, import and transport of viable grains for food and feed production (such as EU and Japan) are the main source of uncontrolled dispersal of these plants (see chapter below). Interestingly, some populations seem to be selfsustaining and can persist without additional gene flow (spillage) at Japanese harbours (Mizuguti et al. 2011; Katsuta et al., 2015). Similar findings are also reported from Canada (Warwick et al., 2008 and Knispel and

McLachla, 2010). This is a strong indication that fitness of these transgenic plants was underestimated. One possible explanation for the higher fitness of transgenic, glyphosate resistant oilseed rape are the findings from Fang et (2018) which show that enhanced fitness is triggered by the EPSPS enzyme also in a glyphosate-free environment.

Table 3 gives an overview of unexpected effects that impact fitness in rice after genetically engineered plants are introgressed with other genetic backgrounds.

Table 4 gives an overview of unexpected effects in oilseed rape that impact fitness after genetically engineered plants are introgressed with other genetic backgrounds or which unexpectedly emerged in persisting transgenic populations.

Table 3: Unexpected effects that impact fitness in rice after genetically engineered plants are introgressed with other genetic backgrounds.

Plant / hybridisation with ...	Trait/ Genes Inserted	Findings	Reference
insect-resistant rice / weedy rice	CpTI, Bt/CpTI	Enhanced relative performance of the crop-weed hybrids, taller plants, more tillers, panicles, and spikelets per plant, as well as higher 1 000-seed weight, compared with the weedy rice parents. Seeds from the F1 hybrids had higher germination rates and produced more seedlings than the weedy parents.	Cao et al., 2009
Glyphosate resistant rice / weedy rice	EPSPS	Transgenic F2 crop–weed hybrids produced 48–125% more seeds per plant than nontransgenic controls. Transgenic plants also had greater EPSPS protein levels, tryptophan concentrations, photosynthetic rates, and per cent seed germination compared with nontransgenic controls. Findings suggest that overexpression of a native rice EPSPS gene can lead to fitness advantages, even without exposure to glyphosate.	Wang et al., 2014
insect-resistant rice / weedy rice	Bt/CpTI	Transgenes can significantly alter the segregation distortion pattern in hybrid progeny, particularly the direction of segregation deviated to different parents. Transgenes with strong selection advantages may have evolutionary impacts on hybrid progeny by changing their pattern of allelic segregation distortion after introgression from transgenic crops to wild relatives through hybridization.	Yang et al., 2014
insect-resistant rice / weedy rice	Bt	Weedy rice has increased fitness after the introgression of Bt genes from cultivated rice.	Lu and Yang, 2009
insect-resistant rice / wild rice	Bt	Increase of Bt content in some hybrids of wild rice and cultivated rice.	Xia et al., 2016
insect-resistant rice / weedy rice	Bt/CpTI	Lower insect damage and higher fecundity in hybrids.	Yang et al., 2015
Glyphosate resistant rice / weedy rice	EPSPS	Transgenic hybrid lineages showed significantly earlier tillering and flowering, as well as increased fecundity and overwintering survival/regeneration abilities.	Yang et al., 2017

Table 4: Unexpected effects that impact fitness in brassica after genetically engineered oilseed rape is introgressed with other genetic backgrounds or which unexpectedly emerged in persisting transgenic populations.

Plant / hybridisation with ...	Trait	Findings	Reference
Oilseed rape	Changes in oil content	Transgenic modifications for modified oil content (such as a higher content of stearat or laurat) provide oilseed rape with fitness advantages.	Claessen et al., 2005
<i>Brassica rapa</i> / Bt oilseed rape	Bt	<i>B. rapa</i> plants that were crossed with Bt oilseed rape produced 1.4 times more seed than the wild type.	Vacher et al., 2004
Oilseed rape	Herbicide tolerance	The properties of some feral transgenic oilseed rape plants seem to have changed, e.g. by showing higher growth and becoming perennial. Climate conditions may be an impact factor for these effects.	Kawata et al., 2009
Oilseed rape	Herbicide tolerance	Populations of transgenic plants can persist in the environment without additional gene flow or factors for specific selection.	Mizuguti et al. 2011; Katsuta, et al., 2015; Warwick et al., 2008; Knispel and McLachlan, 2010

It should be noted that some of these effects might emerge mostly in the first generation due to the effects of hybridisation. As a result, these effects might not be, or might only partially be, inherited in the following generations. Nevertheless, they are relevant in this context since these effects might occur reiteratively and also become cumulative.

2.4 Changes in fitness can be triggered by genome x environmental interactions.

It is well known that unintended effects in GE plants can be triggered by changing environmental conditions or biotic and abiotic stressors (Fang et al., 2018; Matthews et al., 2005; Meyer et al., 1992; Then & Lorch, 2008; Trtikova et al., 2015; Zeller et al., 2010 ; Zhu et al., 2018). There are several reasons why genetically engineered plants show unexpected effects in their interaction with the environment. Specific attention should be given to the genetic functional stability of the inserted DNA. Unlike other organisms, genetically engineered crops inherit technically altered DNA in their cells that does not emerge from evolutionary mechanisms. Many gene constructs are composed from elements such as promoters and stop codons that are not subject to the natural self-regulation of gene expression in the plant cells. Under the conditions of climate change or in interaction with other stress factors and combined with various genetic backgrounds, unexpected effects can emerge in the genetically engineered crops that may pose risks for the environment and ecosystems.

As mentioned, Fang et al. (2018) showed that higher fitness does occur in genetically engineered glyphosate resistant plants in a glyphosate-free environment. They also describe how environmental stressors such as heat and drought can enhance these effects. In *Arabidopsis* producing additional EPSPS enzymes, they observed significantly increased seed germination ratios when transgenic seeds were exposed to heat and drought stresses, although no differences were found in seed germination among different lines when seeds were exposed to normal temperatures. They discuss that this effect is caused by interference of the EPSPS enzyme with the auxin metabolism which can

promote seed germination and plant growth under abiotic stresses.

In addition, several studies with genetically engineered plants such as petunia, cotton, potato, soybean and wheat have reported unexpected reactions to environmental stress conditions (see Table 5). Some of these effects might enhance fitness: for example, there are several findings showing that the Bt content in the plants is influenced by environmental conditions (Table 5).

In addition, specific environmental conditions such as high pressure from pest insects or spraying with the complementary herbicides, can also become a significant driver for enhancing the fitness of the plants, their hybrids and offspring.

Table 5: Examples of unexpected effects in genetically engineered plants due to genome x environmental interactions

Plant	Trait	Findings	Reference
Wheat	Resistance against the fungus powdery mildew	Two of four genetically engineered lines showed up to 56% yield reduction and a 40-fold increase of infection with ergot disease <i>Claviceps purpurea</i> compared with their control lines in the field experiment	Zeller et al., 2010
Oilseed rape	Herbicide tolerance	Perennial growth	Kawata et al., 2009
Maize	Insecticidal	Changes in proteome	Agapito-Tenzen et al., 2013
Potato	Different experimental traits	Stress resistance	Matthews et al., 2005
Petunia	Colour of flowers	Methylation	Meyer et al., 1992
Maize	Insecticidal	Bt content higher or lower due to environmental impact factors such as fertilizer, soil quality, pesticide application and climate	Then & Lorch, 2008
Maize	Insecticidal	Environmental stress can cause unexpected patterns of expression in the newly introduced DNA	Trtikova et al., 2015
Arabidopsis	Herbicide tolerance	Drought and heat stress caused increased seed germination ratios	Fang et al., 2018
Cotton	Insecticidal	Bt content is influenced by environmental conditions and genetic backgrounds.	Adamczyk & Meredith, 2004; Adamczyk et al., 2008; Beura & Rakshit, 2013; Chen et al., 2004; Chen et al., 2012; Luo et al., 2008; Wang et al., 2015; Zhu et al., 2018

3. Other biological characteristics and environmental interactions of relevance

Changes in fitness of the GE crop plants is considered by EFSA to be the only or most relevant issue when it comes to risk assessment of “persistence and invasiveness including plant-to-plant gene flow”. However, this is not the only problem that might arise from persistence and self propagation of GE crops in the environment. In the following we give three examples to exemplify other relevant issues:

(1) Bt producing plants such as cotton (Wegier et al., 2011) or poplar trees (Hu et al., 2017) are examples of GE plants that can persist and propagate in the environment (see also Bauer-Panskus et al. 2013). These plants are likely to show higher fitness compared to its wild relatives under selection pressure posed by Bt-susceptible insects feeding from these plants. But beyond the question of EF, there are other risks that need to be considered such as long term exposure of ecosystems to Bt toxins produced by the GE plants: The Bt toxins will not only be taken up by insects feeding from these plants, but material from the plants such as pollen, seeds, leaves and roots can be distributed in a wider environment: further, the Bt toxins can for example also be secreted by the roots (Saxena et al., 2002). The effects triggered by the Bt toxins might be subtle (see for example Campos et al., 2018; Seide et al., 2018) but large scale and long term exposure might cause these effects to increase substantially, cause changes in insect populations and disturb the functions of the associated ecosystems. The problems in assessing these long term effects are also reflected by Stewart et al. (2003) and Andow & Zwahlen (2006) who refer to the potential impact of the intended traits if transferred into wild populations. It should be taken into account that the Bt content in the plant material can also be influenced by environmental conditions or genetic backgrounds in the offspring (Trtikova et al., 2015).

(2) Life forms interact with the environment via multiple bio-chemical pathways. In plants, these pathways for example include signaling and communication with other plants, microorganisms and insects (see for example Schaefer & Ruxton, 2011; Choudhary et al., 2017). There are various compounds involved such as volatile substances, other secondary metabolites and biologically active compounds. The interactions with the environment encompass the closer (associated microbiomes) or wider environments (such as food webs, predators, beneficial organisms).

Since natural mechanisms such as gene regulation and heredity can be circumvented with modern technical tools of genetic engineering (such as transgenesis and genome editing), experiences of interactions with the environment gained from conventional plant breeding cannot simply be extrapolated to GE plants. For example, da Silva et al (2016) and Wallace et al. (2018) show that differences in the associated microbiomes of transgenic plants are not only influenced by environmental impact but to some extent also impacted by the plant genetics. Therefore, environmental risk assessment of GE organisms should include potential (intended and unintended) changes of these signaling pathways since they can substantially disturb or even disrupt the functions of ecosystems.

These risks are especially relevant in cases in which the plant’s metabolic pathways are changed to, for example, make plants more resistant to stress conditions or to increase yield. Metabolic pathways which interfere with the plants’ growth, stress resistance or plant composition very often are multifunctional and complex. Under these circumstances, any risk assessment has to be driven by the hypothesis that the biological characteristics of the plants as a whole are changed by the

genomic intervention (such as transgenesis or genome editing), including its signalling with the wider or closer environment.

(3) Changes in plant composition causing an altered nutritional quality can cause specific disturbances in the ecological systems via the associated food webs. Since plant composition can be altered by methods of genetic engineering to a much larger extent compared to conventional breeding, existing experience cannot simply be extrapolated to GE plants (derived from transgenesis or genome editing). Therefore, environmental risk assessment of GE organisms should include potential (intended and unintended) changes of the plant composition and their effects on ecosystems. For example, Colombo et al. (2018) show risks for food webs that could result from the extensive cultivation of genetically engineered plants such as oilseed rape with enhanced omega 3 fatty acids: the fatty acids in the plants could, for instance, change the growth and fecundity of the organisms that feed on them. Such effects could be carried forward into the food chain.

The examples above show that plants which can persist and spontaneously propagate in the environment pose substantial challenges for risk assessment, even in cases where the fitness of the plants is not changed.

4. Problems assessing long term and next generation effects

When it comes to the environmental risk assessment (ERA) of genetically engineered plants, its robustness and reliability will be influenced substantially by the question of whether the plants can persist in the environment, if they can spontaneously propagate and / or if gene flow with domesticated and / or wild relative plants can be established leading to viable offspring either in agricultural or semi-natural and natural habitats. The answers given to these questions will substantially impact problem formulation, hazard identification and characterisation as well as the exposure characterisation and final risk characterisation. Very generally stated, if genetically engineered plants can persist in the environment and / or if gene flow with domesticated and / or wild relative plants can be established leading to viable offspring, the uncertainties will increase and the risk assessment will face more complex questions than is the case with plants that cannot persist and cannot establish such gene flow.

There are fundamental differences in the risk assessment of genetically engineered plants that are grown for just one period of vegetation in the fields compared to those being produced each year. If just grown for one season, this enables the company to check the seeds in regard to their most relevant economic characteristics before they are planted in the fields. However, any volunteer hybrids and or GE offspring do not undergo any additional quality or safety check before they appear in the fields. This is especially relevant in cases where self sustaining populations of GE plants get established, no matter whether they are established within the production systems or beyond. For example, Jenczewski et al., (2003) explain:

“transgenes may have different effects in another genetic background (pleiotropy and/or epistasis) or environment. Second, increased fitness of transgenic crops can increase the likelihood of introgression over evolutionary time by making it easier for crop individuals to persist in natural communities. (...).”

Fang et al. (2018) and Beres et al. (2018) also point out this problem. For example, Fang et al. (2018) state:

“Therefore, environmental impact caused by introgression of a transgene overexpressing EPSPS from GE glyphosate-tolerant crops into their wild/weedy relatives should be

thoroughly assessed, even in the glyphosate-free environment. Further studies including hybrid descendants of transgenic crops overexpressing EPSPS with their wild relatives should be conducted to provide more evidence for the potential ecological impact.”

The problem can also be exemplified in the case of genetically engineered rice: if GE rice showing tolerance to biotic or abiotic stress factors can pass its characteristics to weedy rice, this might cause weed problems for farmers to increase (see for example Lu & Snow, 2005). Further, in regard to the offspring and hybrids of these plants, further levels of complexity have to be taken into account:

- Gene flow between the domesticated and the weedy rice can trigger EF or other biological effects emerging within in the next generations even if no such characteristics were observed in the original events. Especially in hybrids, the added genetic material will be introduced in various genetic backgrounds, which can cause unintended effects (see Table 3).
- If gene flow is reiterated as described for weedy and domesticated rice (Vigueira et al., 2013; Kanapeckas et al. 2016), new combinations of genetic material might occur in the following generations, such as spontaneous stacked events with, for example, resistance to several biotic or abiotic stressors. These combinations can trigger enhanced fitness or other biological effects with much stronger impacts than observed in the original event.
- Finally, if the plants persist over a longer period of time and / or spread to various receiving environments, the likelihood that EF or other biological effects are triggered by environmental x genome interaction (see Table 5) may increase. Relevant effects might be absent in the first generation(s) and only emerge after several crossings and / or under specific environmental circumstances.

Thus, if the plants can persist in the environment and / or if gene flow with domesticated and / or wild relative plants can be established, leading to viable offspring, hazard identification and characterisation has to include several and complex scenarios which also have to deal with hazards that are not predictable just from the data of the original events.

This problem of increasing complexity is especially relevant for plant species that have a high potential to persist in the environment and/ or to spread into domesticated or native relatives. Table 6 lists some of the plant species that are used for genetic engineering and are known for their potential to persist and perform gene flow in the regions where they are cultivated (also see Ellstrand, 2018)

Table 6: Examples for (potential) gene flow from GE plants to wild relatives species

Species	Potential to persist in the environment, independently from cultivation	Estimated potential for spontaneous gene flow to relatives (per region)	Observation of gene flow from genetically engineered plants (per region)
Alfalfa	Wild species are known to exist in Europe and to hybridise with cultivated varieties (Prosperi et al., 2006)	High potential to cross with wild relatives (Jenczewski et al., 1999)	No data available
Creeping bentgrass	High potential, listed as a weed in parts of the US ¹	Creeping bentgrass is part of a species complex, in which species are able to hybridise	Parts of the US (Oregon, Idaho) (Zapiola et al., 2008; Watrud et al., 2004).

¹ *A. stolonifera* is listed as a weed in the West (<http://plants.usda.gov/java/invasiveOne?pubID=WSWS>) and in the Middle West (Nebraska and the Great Plains) of the US (<http://plants.usda.gov/java/invasiveOne?pubID=NEGP>).

		with each other (Rotter et al., 2010). According to Wipff (2002), some of the hybrids are so common that they have specific names.	
Cotton	Low potential in Europe. Higher potential in countries of origin (such as Mexico).	Genetic material can spread and persist into regional varieties in country of origin.	Gene flow / contamination observed in wild cotton in Mexico (Wegier et al., 2011).
Eggplant	High potential in country of origin such as India and Bangladesh (Davidar et al., 2015).	Genetic material can spread and persist into regional varieties in country of origin.	No data available
Maize	In the field: High potential in country of origin or other countries with mild winters such as South Africa (Iversen et al., 2004) and Philippines (Greenpeace, 2013). The occurrence of some genetically engineered maize plants outside cropped areas has been reported in Korea due to grain spillage during import, transport, storage, handling and processing (Kim et al., 2006; Lee et al., 2009; Park et al., 2010)	In Europe, there is some potential for gene flow due to the occurrence of wild relative teosinte (for example Spain) (Trtikova et al., 2017). Genetic material can spread and persist into regional varieties in country of origin (Dyer et al., 2009) and in other countries (Greenpeace, 2013).	Gene flow / contamination observed in regional varieties in Mexico (Quist and Chapela, 2001; Dyer et al., 2009) and Philippines (Greenpeace, 2013).
Oilseed rape	Also outside the agricultural system, both medium potential in region of origin (Europe) as well as other regions (North America, Japan and Australia) (Bauer-Panskus et al., 2013)	Genetic material can spread and persist into wild relatives in Europe and other regions.	Persistence for several generations described in Japan, Canada, the US and Europe (for overview see Bauer-Panskus et al. (2013))
Poplar	Poplar species hybridise with each other frequently (Aas. n.d.). In the course of evolution, some hybrids have become separate species.	Genetic material could spread and persist into wild relatives in Asia and Europe and other regions (Bauer-Panskus et al., 2013)	A first study of gene flow from Bt poplar plantations in China was provided in 2017 (Hu et al., 2017)
Rice		Can spread in circles between domesticated varieties in the fields and weedy rice besides the fields (Lu & Snow, 2005)	Contamination from field trials with genetically engineered rice in China (Greenpeace, 2013) led to long-term adventitious presence of the transgenes in rice imported to the EU.

Potential harm can be caused by enhanced weediness, invasiveness or disruption of ecological networks. Relevant causes and scenarios have to take into account genome x environment interaction, effects emerging from genetic background, next generation effects, epigenetic effects and effects of hybridisation with wild relatives. These effects do not depend on specific scenarios

such as introgression into wild populations or spreading beyond sites of agricultural production. For example, if the transgenes persist and spread in regional varieties of maize in Mexico, which is one of the countries of origin (Serratos-Hernández, 2009), any of the effects as mentioned above are relevant for the overall risk analysis.

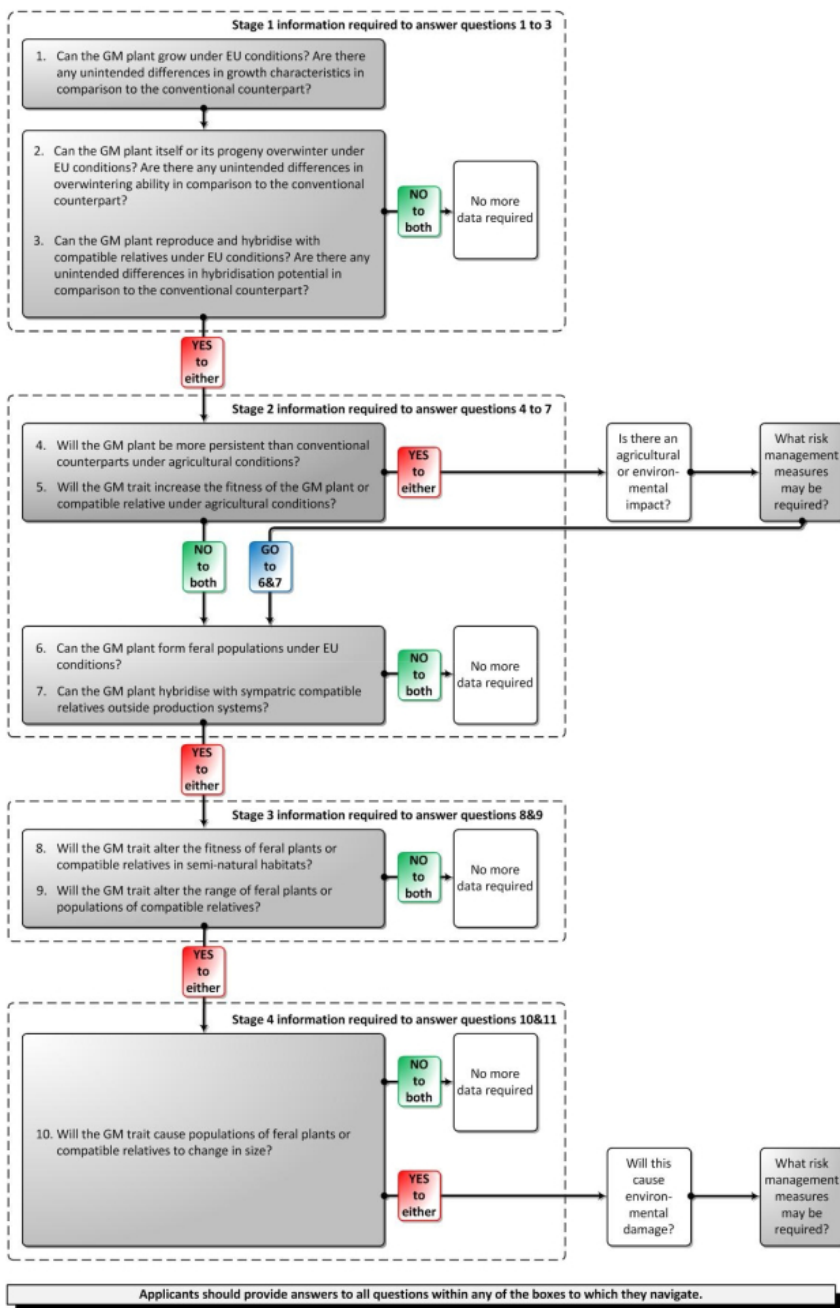
The same is true for potential gene flow from and to teosinte as being observed in the fields in Spain (Trtikova et al., 2017): these plants are wild relatives (ancestors) of cultivated maize. Depending on the subspecies of teosinte, gene flow is more or less likely to occur. The subspecies occurring in Spain has not been fully identified and seems to be a hybrid between maize and teosinte. Its actual potential for gene flow with maize in the fields is not known (Trtikova et al., 2017).

Devos et al. (2018) acknowledge that currently there is no “*information of the expression of the transgenes in the hybrid plants*”. These experts do not deem such data to be necessary. Instead, they simply state that a “*worst-case assumption is that any teosinte × GM maize hybrids will express/manifest the traits that the transgenes confer*”. Thus, experts like Devos et al (2018) assume that once the transgenes have escaped to teosinte they will somehow preserve the intended biological trait originally inserted. They seem to think of the transgene as an inert BioBrick, which has a predictable function that is independent of the rest of the organism and its interaction with the environment. This is wrong. Since the effects caused by gene flow largely depend on interactions with the environment and the genetic backgrounds of the plants, the characteristics of volunteer offspring and next generation effects cannot be predicted from the characteristics of the original event.

Devos, who is the leading author of Devos et al (2018), is also a member of the GMO Unit at EFSA. It has to be emphasized that in general, EFSA has no adequate guidance and methodology to perform risk assessment of changes in the plants’ fitness that can emerge from unintended genomic effects, next generation effects and / or effects that might be triggered by specific environmental conditions. The staged approach as suggested by EFSA (2010, see Figure 1) fails to produce sufficiently robust and reliable results, because

- (1) the potential impacts caused by the offspring of the GE plants are mostly assessed on the level of the original event
- (2) in terms of potential hazards, only changes in fitness leading to exacerbating weed problems, displacement or even extinction of native plant species are taken into account.

Figure 1 (derived from EFSA 2010, page 43): Questions defining the different stages of risk assessment concerning persistence and invasiveness of a GE plant itself, or any of its introgressed relatives, as a result of vertical gene flow.



As a result, there are considerable gaps in the risk assessment as performed by EFSA. In general, if genetically engineered plants and / or their altered genetic material are able to persist in the environment and to introgress into domesticated or wild relatives, risks emerging from genome x environment interactions or from crossing / hybridisation are very likely to escape risk assessment as performed by EFSA.

5. Regulatory options to take into account spatio-temporal complexity

Beyond the specific gaps in risk assessment as performed by EFSA (2010) there are some more fundamental problems with the ERA in respect to GE organisms which can persist and spontaneously propagate in the environment, especially if gene flow to wild relatives cannot be excluded. If the spatio-temporal dimension cannot be determined, the problem formulation including hazard identification, hazard characterisation and exposure characterisation might not be sufficiently defined reach conclusions about the environmental risks. To assess the problem of spatio-temporal complexity, the following questions should be answered (see Table 7):

- Can genetic stability be controlled in following generations?
- How can genetic diversity in the wild population of the same species be taken into account?
- Will there be any gene flow to other species?
- How can the population dynamics and life cycle aspects of the wild species be integrated?
- Can the receiving environment be defined in regard to relevant interactions and confined in regard to potential spread?

Table 7: Overview of relevant questions for risk assessment of genetically engineered plants which can persist and spontaneously propagate and perform gene flow in the environment.

Question	Relevance	Which methodology is available?
(1) Can genetic stability be controlled in following generations?	Self-replication and environmental as well as epigenetic effects can lead to emergence of next generation effects not observed in the first generation.	Several generations should be observed under a wide range of defined environmental conditions. The outcome has to be put in context to questions (2) and (3).
(2) How can genetic diversity in wild populations of the same species be taken into account?	In most cases a high degree of genetic diversity exists in natural populations. These heterogeneous genetic backgrounds can trigger unexpected effects not observed in domesticated populations.	Genetic diversity is reduced in domesticated plants and might only represent a small selection of the genetic diversity within wild populations of the same species.
(3) Will there be any gene flow to other species?	If gene flow is possible and hybrid offspring are viable, the resulting organisms have to be seen as new events that have to be assessed separately from the original GE organisms.	It might be possible to perform crosses under controlled conditions. Results have to be put in context with question (1) and (2).
(4) How can population dynamics and life cycle aspects of the target species be integrated?	For example, bottlenecks in the population dynamics can have a significant impact on tipping points within the populations.	Large scale population effects can be modelled, but empirical investigations are difficult. Further, any results have to be interpreted in the light of question (1) and (2).
(5) Can the receiving environment be defined in regard to relevant interactions and confined in regard to potential spread?	Adverse effects can emerge from interaction with closer (associated microbiomes) or wider environments (such as food webs, predators, beneficial organisms). Complex interrelations (such as signalling pathways) have to be taken into account.	These aspects have to be assessed case by case and step by step. In most cases, long-term, cumulative and combinatorial effects cannot be tested or investigated <i>ex ante</i> .

Table 7 indicates that in many cases significant uncertainties remain and some unknowns might prevail that make the risk assessment inconclusive: the multiplex interrelations with the closer and

wider environment pose a real challenge for the risk assessor. While genetic stability over several generations might be demonstrated in domesticated varieties under normal field conditions or greenhouse cultivation, genome x environmental interactions and introgression into heterogeneous genetic backgrounds still can trigger unpredictable next generation effects. Whatever the case, the biological characteristics of the original events cannot be regarded as sufficient to predict all relevant effects that can emerge in the next generations, and in interaction with the receiving environments.

In general, if the spatio-temporal dimension cannot be defined, risk assessment of genetically engineered organisms has to consider evolutionary dimensions. The problem: evolutionary dynamics combine large numbers of individuals on the population level and singularities on the molecular scale. Thus, evolutionary processes make it possible to turn events with a low probability of ever happening into events that may feasibly happen (Breckling, 2013). Under these conditions, for example, the fitness of new genomic constituents cannot be calculated in absolute terms; it will depend on the environment and future changes.

Risk assessors and risk managers need to solve the problems of how to come to robust conclusions and make reliable decisions that take the precautionary principle into sufficient consideration. This is similar to a problem dealt with in EU regulation of chemicals and pesticides. In this context, the spatio-temporal dimension also plays a decisive role. For example, Recital 76 of EU Regulation 1907/2006 (REACH) addresses the issue: “Experience at international level shows that substances with characteristics rendering them persistent, likely to bioaccumulate and toxic, or very persistent and very likely to bioaccumulate, present a very high concern, while criteria have been developed allowing the identification of such substances.” Consequently, criteria to identify persistent, bio-accumulative and toxic, as well as very persistent and very bio-accumulative chemical substances, are defined in ANNEX XIII of the regulation EU Regulation 1907/2006.

Further, EU Regulation 1107/2009 integrates the criteria of POP (persistent organic pollutant), PBT (persistent, bio-accumulative, toxic) and vPvB (very persistent, very bio-accumulative) into the regulatory decision making process. These criteria function as so-called cut-off criteria: in essence, the approval process should not proceed if the substance is “POP”, “PBT” or “vPvB”. In this context, it is important that the chemical substances are not only assessed in regard to their toxicity but also, more generally, in regard to their “fate and behaviour in the environment” (EU Regulation 1107/2009, Annex II, 3.7.) which gives decisive weight to the spatio-temporal dimension. If a substance is regarded as very persistent and very bio-accumulative, there might still be some uncertainty or non-knowledge in regard to its actual long-term adverse effects. Nevertheless, according to EU Regulation 1107/2009, it cannot be approved. For example, Annex II, point 3.7.3 of EU Regulation reads: “An active substance, safener or synergist shall only be approved if it is not considered to be a very persistent and very bioaccumulative substance (vPvB).”

The way in which cut off criteria were established for chemicals could also be useful as a model for the risk assessment of GE organisms. Similarly to EU regulation of chemicals, the fate and behaviour of the organisms in the environment would be a crucial aspect. Persistence can be self sustaining or be dependent on geneflow from cultivation or spillage of genetically engineered plants. If volunteer generations occur and / or if geneflow to wild relatives is expected, the biological characteristics of the next generation might deviate substantially from the original event in regard to fitness, composition and / or environmental interaction, and risk assessment will suffer substantially from major uncertainties. Therefore, if it were known that GE organisms could escape 'spatio-temporal controllability' by reproducing within natural populations without any effective control of spread or persistence, then the authorisation process could not proceed and the release of

the GE organisms could not be allowed.

Thus, in effect, GE organisms could neither be approved nor released, if actual long-term adverse effects could not be determined in detail. How then can criteria be developed for the risk assessment of genetically engineered organisms that are sufficiently well defined and applicable in the approval process, as well as that take into account uncertainties and limits of current knowledge? As described above in the context of chemical substances, the cut-off criteria are defined so that known characteristics of the substances are used to integrate uncertainties around actual long-term impacts into decision making.

By analogy, the criteria applied in the risk assessment of genetically engineered organisms should be as clear and well defined as possible. Well-established scientific criteria from three areas of knowledge should be taken into consideration: (1) the (natural) biology of the organisms (2) their (naturally) occurring interactions with the environment (biotic and abiotic) and (3) the intended biological characteristics (traits) inserted through genetic engineering. These elements should be combined to establish an extra step in the risk assessment of GE organisms, aimed at assessing 'spatio-temporal controllability'. Table 8 provides an overview of some relevant details that can be used to evaluate 'spatio-temporal controllability' in these three categories.

Table 8: Some specific issues relevant for the assessment of 'spatio-temporal controllability' (vertical reading)

Biology of the species (wildtype)	Interactions of the organisms with the environment (wildtype)	The intended biological characteristics of the GE organism
Potential to persist and propagate	Interactions within the ecosystem: <ul style="list-style-type: none"> - position in the food web - closely associated organisms (microbiome, parasites, symbiotic organisms) - within the wider environment (such as beneficial insects, soil organisms, protected species) 	How can genetic stability be controlled in following generations after the release?
Population dynamics and life cycle	Impact of biotic stressors e.g. pests and pathogens.	Does the GE trait impact the fitness of the organisms?
Potential to spread beyond fields / into different ecosystems	Occurrence of abiotic stressors such as climate conditions (whole life cycle)	Does the trait impact the composition of biologically active compounds?
Potential for reproduction with wild populations of the same species	Role and function in energy- and nutrient-cycle	Can the persistence of the organisms be determined if necessary?
Genetic diversity in wild populations of the same species		
Potential for gene flow to other species		

This approach uses specific 'knowns' to decide upon 'known unknowns' (such as next generation effects and genomic x environmental actions). It is assumed, the criterion of 'spatio-temporal controllability' can inform regulatory decision making even in the light of major uncertainties emerging from the spatio-temporal dimension. This can be seen as the equivalent of cut-off criteria such as "PBT" and "vPvB" that are anchored in the EU regulation of chemical substances.

It should, however, be recognised that the assessment of 'spatio-temporal controllability' is just a step within risk assessment and not a replacement for it. Risk assessment might well be terminated

due to the assessment of 'spatio-temporal controllability', but if it proceeds, all other steps and criteria still have to be applied.

In general, the assessment of 'spatio-temporal controllability' is suitable to produce results which are meaningful and allow the application of cut-off criteria within the process of risk assessment. For example, the authorisation process should not proceed and the release of the GE organism should not be allowed if it is known that the GE organisms are able to escape 'spatio-temporal controllability' due to propagation in natural populations, with no effective control of spread or way of preventing persistence in the environment.

As far as the role of the risk manager in the EU is concerned, it should be acknowledged that applications for releases of GE organisms that lack spatio-temporal control can already be rejected, even without an additional and specific step in risk assessment as suggested. In regard to spatio-temporal control, EU Directive 2001/18 could be used as a legal basis to take the relevant decisions: according to Krämer (2013), spatio-temporal control is a necessary prerequisite to enable the precautionary principle. Directive 2001/18/EC foresees the possible withdrawal of authorisation in case of urgency (Article 23) or the potential to reject renewal of the authorisation after ten years (Article 17). Therefore, Krämer comes to the conclusion that "Where there is, in a concrete case, a likelihood that genetically modified plants or animals cannot be retrieved, the legal obligation to ensure that any release must be 'safe' requires the refusal to authorize such releases." (Paragraph 250). However, Krämer also shows that there are significant uncertainties in the implementation of EU regulation that require further attention and which for example could be ruled out by additional steps in risk assessment as proposed by this report.

The assessment of spatio-temporal controllability as suggested is not an assessment of specific risk *per se*. Rather, it is related to the overall conclusiveness of the risk assessment. Therefore, being aware of the implications of Directive 2001/18, Art. 1 (emphasizing the precautionary principle) Art. 17 and Art. 23, the requirement to demonstrate spatio-temporal controllability could be introduced at the beginning of the approval process as part of the data check before detailed risk assessment starts. If spatio-temporal controllability is not demonstrated, the application should be rejected. This might not be appropriate in all cases. Thus, EFSA could also introduce the assessment of spatio-temporal controllability within the environmental risk assessment. So far, issues such as gene flow are already included. In its opinion, EFSA could point out the relevant uncertainties and uncertainty in regard to spatio-temporal controllability. If spatio-temporal controllability cannot be demonstrated, the risk assessment cannot be concluded. As a consequence, the overall approval process should be postponed or terminated by the risk manager. There are already several examples of inconclusive EFSA opinions that stopped or substantially delayed the approval process e.g. EFSA opinions on maize 98140 (EFSA 2013a) and maize 3272 (EFSA 2013b). Furthermore, in an EFSA presentation from 2018 (EFSA 2018), the following reasons were given for rendering scientific opinions of EFSA inconclusive: (i) Lack of sufficient data to conclude the risk assessment (e.g.) (ii) Lack of toxicological study (iii) Incomplete set of data linked to genotoxicity (iv) Lack of complete set of compositional data (v) Lack of data to characterise the process / the product (vi) Lack of data on efficacy (vii) Waiving of data and (viii) Inadequate study design.

6. A case study: Risk assessment of GE oilseed rape

The advantages of an extra step in the risk assessment of GE organisms, aimed at assessing ‘spatio-temporal controllability’, can be exemplified in the case of risk assessment of spontaneous feral populations which can emerge from spillage of GE oilseed rape, meant for import:

MON88302 is a genetically engineered herbicide-resistant oilseed rape developed by Monsanto, which is designed to withstand even higher dosages and even more frequent applications of glyphosate than before. The authorisation granted in 2015 also concerns viable whole kernels, which can give rise to feral populations after spillage.

Europe is the centre of origin and genetic diversity for the group of *Brassica* plants to which oilseed rape belongs. Thus, there are several wild relatives that can interbreed with oilseed rape (*Brassica napus*). Oilseed rape can spread via pollen and seeds and can be described as a high-risk crop for crop-to-crop gene flow and from crop to wild relatives (Eastham and Sweet, 2002). Further, the seed remains viable in the soil for more than ten years (Lutman et al., 2003). Consequently, oilseed rape has a high potential for establishing volunteer plants even many years after the first sowing. The plants are mostly pollinated by insects such as flies, honey bees and butterflies which can also carry the pollen over many kilometres. Wind is also relevant for pollen drift: The farthest pollen-mediated outcrossing distance measured to date is 26 kilometres, recorded in a field trial with sterile male pollen (Ramsey et al., 2013). Oilseed rape can appear in ruderal populations (i.e. growing on waste ground) along field edges and roadsides. Banks (2014) found that ruderal populations are self-sustaining in a semi-permanent form. According to Munier et al. (2012), herbicide tolerant oilseed rape is a weed. There are weedy forms of *B. rapa* and *B. oleracea*. The wild relative species *Sinapis arvensis*, *Raphanus raphanistrum* and *Hirschfeldia incana* are also considered to be weeds (OECD 2012).

Environmental risk assessment as performed by EFSA requires that spillage from viable kernels of imported products is also assessed (EFSA 2010, page 41):

“It should also consider viable GM plant seeds or propagules spilled during import, transportation, storage, handling and processing that can lead to feral plants that colonize and invade ruderal, semi-natural and natural habitats.”

EFSA (EFSA 2014a) is of the opinion that the import and transport of MON88302 (which they summarise as genetically modified herbicide tolerant – GMHT - oilseed rape), is likely to establish volunteer plants along transport routes and processing facilities. However EFSA does not consider this to be a problem:

“The EFSA GMO Panel confirms that feral GMHT oilseed rape plants are likely to occur wherever GMHT oilseed rape is transported. However, there is no evidence that the herbicide tolerance trait results in enhanced fitness, persistence or invasiveness of oilseed rape MON 88302, or hybridising wild relatives, unless these plants are exposed to glyphosate-based herbicides. Escaped oilseed rape plants and genes introgressed into other cross-compatible plants would therefore not create any additional agronomic or environmental impacts.”

As argued in the EFSA’s opinion (EFSA 2014a), the GMO panel is of the opinion that the occurrence of feral MON88302 oilseed rape resulting from seed import spills is likely to be low, as is the likelihood of gene flow to wild relatives. However, these assumptions can be doubted: in general, the amount of spillage will be largely dependent on the amount of imports, the transport routes and the transport vehicles. The frequency of spillage is likely to increase with a higher

volume of imports. Demands for import might vary over the years and are driven by various markets, not only for use in food and feed but also for energy production.

As the statements from experts of Member States also show (EFSA 2014b), several publications show that spillage from transport can occur in amounts that give rise to populations that can persist in the environment over several years and also that gene flow occurs between these populations and wild relatives. Studies have shown that oilseed rape seed can produce progeny in semi-natural habitats. Feral oilseed rape populations can persist for several years (Pessel et al., 2001; Schafer et al., 2011). While they persist mainly through the soil seed bank (Pivard et al., 2008a; Pivard et al., 2008b), they can in fact constitute transgene reservoirs. Knispel & McLachlan (2010) as well as Warwick et al. (2008) found that feral herbicide-resistant populations became a permanent feature of agricultural landscapes in western Canada. Not only under selection pressure (for example glyphosate treatment for glyphosate-tolerant oilseed rape) but also without it, these populations can grow in number and contribute to gene flows in neighbouring fields (Squire et al., 2011). One possible reason for this is that the EPSPS enzyme which confers resistance to glyphosate also triggers enhanced fitness in a glyphosate-free environment (Fang et al., 2018).

As the example of Japan shows, import can also cause the emergence of self-sustaining populations. Japan is especially relevant in this context because even though transgenic oilseed rape is not commercially cultivated in this country, genetically engineered oilseed rape has been found growing and this has been attributed to imports. The first studies on the presence of transgenic oilseed rape in Japan were published in 2005 (Saji et al., 2005). Plants that proved to be resistant to glyphosate or glufosinate were found in the proximity of ports like Kashima, Chiba, Nagoya and Kobe as well as along transportation routes to industry plants where oilseed rape is processed. Follow-up studies found ruderal populations along further transportation routes (Nishizawa et al., 2010) and in areas close to all other major ports (such as Shimizu, Yokkaichi, Mizushima, Hakata, or Fukushima) (see for example Kawata et al., 2009; Mizuguti et al. 2011). Further, the publication of Mizuguti et al. (2011) came to the conclusion that oilseed rape populations are able to self-sustain over time. Obviously, the percentage of transgenic oilseed rape in ruderal populations is constantly growing. In 2008, 90 percent of all tested plants in the proximity of Yokkaichi port proved to be genetically engineered. In these cases, the additional EPSPS enzyme produced in these plants might also play a crucial role (Fang et al., 2018).

Together with feral oilseed rape populations, transgenic volunteers can open up many opportunities for genetic recombination, stacking of genes, and the evolution of genotypes that could lead to not only an increase in the cost of weed control in the future, but also to phenotypes with new environmental risks such as enhanced invasiveness. For example, new combinations of herbicide resistant traits can emerge such as crossings with 'Clearfield' oilseed rape which is grown in the EU and was made resistant by mutagenesis to an ALS-inhibitor herbicide called imazamox. Oilseed rape could become a multi-resistant weed with a much higher fitness (at least under current agricultural practices) compared to other oilseed rape plants.

There are several findings which concern crossings of wild and domesticated plants that give rise to transgenic offspring. The first transgenic hybrid plants between *B. napus* and *B. rapa* were found in Yokkaichi (Aono et al., 2011). Aono et al. (2006) also detected herbicide tolerant transgenic oilseed rape plants that had hybridised with each other and were thus tolerant to both glyphosate and glufosinate herbicides. Crosses between transgenic plants that give rise to spontaneous stacked events are also reported by Schafer et al. (2011). If these crossings inherit higher fitness, as suggested by Beres et al (2018), the transgenic plants might replace native populations and become weeds which can spread faster in the fields and the environment.

EFSA (2014a) did not request any data on seed dormancy, duration of flowering, number of pollen, viability of pollen nor on any other parameter which is crucial to judge whether the plants have enhanced fitness. Further, it did not assess in detail the impact of a delay in flowering as observed in the transgenic plants. Significant differences, that were observed in seed maturity and lodging, were set aside by EFSA as not of biological relevance and therefore not assessed any further. In conclusion, there are hardly any specific data to assess fitness, persistence or invasiveness of oilseed rape MON88302. In consequence, EFSA (2014a) overlooked or ignored all indications of higher fitness of the transgenic oilseed rape which result from observations in Canada and Japan (see table 4). Meanwhile, these indications are strongly supported by Fang et al. (2018).

Furthermore, EFSA (2014a) only took into account the characteristics as observed in the original event. By assuming that offspring and hybrids would show the same characteristics as the original events, EFSA overlooked publications that indicate unexpected changes in the fitness of transgenic plants that are unrelated to the intended trait: such as Kawata et al. (2009) or Aono et al. (2006) (see Table 4). No crossing experiments with MON88302 were performed to investigate the effects of the transgenes in plants with other genetic backgrounds. It is therefore not possible to predict fitness, persistence or the invasiveness of hybrids from crossing with oilseed rape MON88302.

Genome x environmental interactions were ignored as well. For example, outcrossing into wild species could be enhanced by climate or other environmental change. A higher amount of gene flow has been reported for oilseed rape under extreme climatic conditions (Franks and Weis, 2009). This study shows there was a change in the time for flowering, resulting in matching of flowering between species.

In a possible long term scenario, by the crossing of wild relatives with other transgenic oilseed rape, the resulting transgenic plants could become resistant to one or several herbicides. If such a scenario became reality, the transgenic plants might become a “superweed” with invasive characteristics (this being likely to occur also without the application of the complementary herbicides) that could endanger oilseed rape production in the EU and, if transgenic plants are established outside cultivated fields, also impact wider ecosystems.

In this context, an extra step the in risk assessment of GE organisms aimed at assessing ‘spatio-temporal controllability’ would provide more clarity, transparency and also more reliability in final decision making. As a consequence, no import of viable kernels should be allowed, or effective measures have to be established to prevent any spillage.

7. Conclusions

Genetically engineered plants did not undergo evolutionary processes and they are not derived from existing biodiversity. Therefore, their introduction into the environment, by being grown at a large scale, and products derived from them being introduced into the food and feed chain, is exposing humans and the environment to risks that have no precedent.

We show that risk assessment of genetically engineered plants that can persist and propagate in the environment cannot be reduced to the specific traits and characteristics that are known at the stage of application, but also has to take into account effects that can emerge after some generations, in other genetic backgrounds or under stress conditions.

Furthermore, we show that exacerbating weed problems, displacement or even extinction of native

plant species (EFSA 2010) are not the only risks that might arise from persistence and self propagation of GE crops.

Much more weight has to be given to the assessment of the plant's interactions and biological communication networks such as within the food web, the soil organisms or insects such as pollinators and other organisms. These networks can be disturbed or disrupted by changes in the composition of volatile compounds or biochemical pathways and changes in nutritional quality.

Very generally, in the light of these uncertainties and research gaps, it has to be concluded that at some point, the uncertainties and unknowns in risk assessment will become predominant in comparison to the knowledge available, affecting the ability to conclude on the safety of genetically engineered plants. Consequently, robust and sufficiently reliable risk assessment of GE organisms can only be conducted if it is based on a spatio-temporal dimension that is clearly confined.

Against this backdrop, we recommend establishing 'cut off criteria' in risk assessment that take into account the factual limits of knowledge. It is proposed to introduce these 'cut-off criteria', based on a specific step of 'spatio-temporal controllability' within risk assessment. This new step combines three elements:

- (1) the natural biology of the organisms,
- (2) their naturally occurring interactions with the environment (biotic and abiotic)
- (3) the intended biological characteristics of the GE organism.

The combination of these three elements in one specific, additional step in risk assessment has the advantage of them already being used to some extent in current EFSA risk assessment; many of the details to assess these elements are very well known. If it is known that GE organisms can escape 'spatio-temporal controllability' because they can propagate within natural populations with no effective control of spread or persistence, then the authorisation process cannot proceed and the release of the GE organism cannot be allowed. This concept can be used to delineate some of the boundaries between known and unknowns considered to be crucial. Consequently, this additional step in risk assessment will foster the robustness of risk assessment and can substantially benefit the reliability of decision making within approval processes.

These criteria should not only be applied to applications for commercial cultivation but also to imports that are likely to cause spillage of viable kernels of relevant events / species. In general, the release of genetically engineered plants should not be allowed if their persistence in the environment cannot be controlled in the spatio-temporal dimension.

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