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Escape of genetically engineered organisms and unintentional transboundary movements: Overview of recent and upcoming cases and the new risks from SynBio organisms

Andreas Bauer-Pankus, Sylvia Hamberger,
Mirjam Schumm & Christoph Then 2015
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Overview of recent and upcoming cases and the new risks from SynBio organisms**

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Summary

This report gives an overview of cases of uncontrolled spread and/or gene flow from genetically engineered organisms that can enable them to become established, persist and propagate in the environment, independently of their intended usage. Long-term potentially permanent persistence of the newly introduced DNA in native populations can be a consequence.

In a first global overview, we were able to show that transgenes have escaped, become persistent beyond the fields and sites of their intended release and have also partially moved into wild populations. In this report, we present further evidence that has emerged for some of the previously reported cases (Bauer-Panskus et al., 2014, a, b). The cases concern China (rice), Mexico (maize and cotton), Japan (oilseed rape), South Korea (maize and cotton) and Switzerland (oilseed rape).

New cases of transgene escape concern food plants, grasses, trees and, for the first time, genetically engineered animals, pine trees (USA), new variants of herbicide resistant grasses (USA), alfalfa (USA), maize (Philippines) and genetically engineered mosquitoes (Brazil and Panama). Since there is a general lack of oversight, there are major uncertainties regarding factual gene flow and persistence.

Future cases of transgene escape are likely to concern Bt eggplant (India / Bangladesh), genetically engineered salmon (North America), olive flies (Europe) and eucalyptus trees (Brazil).

To add to this scenario, there are increasingly serious risks associated with new methods currently being used in synthetic gene technologies to produce organisms that, for example, can act as gene drives. The pattern of heredity is manipulated in these organisms in such a way that it enables the new synthetic DNA to spread much more rapidly than normal into native populations.

These cases seem to be very relevant for finding an answer to the question raised by the secretary of the Convention on Biological Diversity (CBD), who is calling for information ¹

“on actual cases of unintentional transboundary movement and case studies related to their existing mechanisms for emergency measures in case of unintentional transboundary movements of living modified organisms that are likely to have significant adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, including information on existing rapid alert mechanisms and monitoring systems.”

It appears that several of the cases listed have the potential for spontaneous, unintentional transboundary movement. Further, the cases as documented are mostly relevant in regard to the conservation of biological diversity.

In addition, it is very difficult to predict the long-term ecological impact of transgenes that escape spatio-temporal control. Long-term, evolutionary processes make it possible for events with a low probability of ever happening to turn into events that may feasibly happen (Breckling, 2013). Consequently, performing reliable risk assessment may no longer be possible.

So from a regulatory point of view, the spatio-temporal control of genetically engineered organisms is a fundamental precondition for any reliable risk assessment. Whilst from the perspective of the precautionary principle, the release of any genetically engineered organisms that cannot be controlled in their spatio-temporal dimension should be prevented.

¹ <https://www.cbd.int/doc/notifications/2015/ntf-2015-002-bs-en.pdf>

In awareness of the enormous potential of synthetic biology or synthetic gene technologies (genome editing), the obvious need for regulation seems more pressing than ever. In relation to biodiversity, this implies that the number of different technically derived organisms being released into the environment within the next few years could increase dramatically. Therefore, the likelihood of negative consequences will also increase – especially if these organisms escape spatio-temporal control.

We are not aware of any internationally binding framework that could be used to enforce a global restriction or ban on the release of organisms derived from genetic engineering or synthetic biology if spatio-temporal control cannot be established, even though there might be spontaneous transboundary movement. Current regulatory frameworks and instruments are not sufficiently robust to prevent gene flow from transgenic or synthetic organisms to native populations.

We are at a crossroads. There is no doubt that in the age of the “Anthropocene”, biodiversity has already been severely impacted by human activities such as those in agriculture and land use. Gene flow from transgenic organisms and/or from organisms derived from synthetic gene technologies into the environment and native populations, is adding an extra level of risk to biodiversity. If we do not manage to stop the uncontrolled spread of genetically engineered organisms, all future generations will have to deal with new uncertainties, risks and long-term impacts created by current short-term economic interests and flaws in international and national regulation.

Therefore, there is a pressing need for international and national political action. Starting with the recommendations made by Bauer-Panskus et al. (2013), we propose the establishment of international regulations that allow the release of genetically engineered or synthetic organisms to be banned if

- › they can persist and invade the environment if they unintentionally escape their containment.
- › there are major doubts that they can be withdrawn from the environment within a reasonable period of time if this is so required in cases of urgency.
- › it is already known that they will persist or show invasive behaviour after release into the environment.

1. Introduction

Ever since genetically engineered organisms were first introduced into agriculture there have been concerns that they could escape into the environment and spread into native populations. In 2013, we reviewed case studies of the cultivation-independent establishment of genetically engineered plants in the environment (Bauer-Panskus et al., 2013 a,b).

The publications covered the following examples of genetically engineered plants:

- › Creeping bentgrass in the US
- › Cotton in Mexico
- › Maize in Mexico
- › Oilseed rape in Canada, US, Japan, Australia and Europe
- › Virus resistant papaya in Thailand
- › Bt poplar in China
- › Bt rice in China

The case studies focused on the presence of feral populations of genetically engineered plants and introgression from transgenic plants into wild populations, as well as into landraces.

In some cases in North and Middle America, it has to be assumed that transgenes from species such as bentgrass, oilseed rape and cotton have already escaped permanently, or at least for an unknown period of time, into the environment, and also moved into wild populations.

There are several causes for the spread of transgenes: Apart from commercial cultivation and experimental field trials, some case studies showed that the uncontrolled dispersal of genetically engineered plants can also be due to the import of viable grains for food and feed production.

This review made several recommendations for political action:

- › measures should be put in place immediately to as far as possible stop any further uncontrolled spread of genetically engineered organisms into the environment;
- › comprehensive regulation should be established to strengthen the precautionary principle, and
- › the release of genetically engineered organisms should not be allowed if they cannot be retrieved from the environment.

To underpin these recommendations, environmental organisations from many regions published a joint call to the contracting parties of the international Cartagena Protocol on Biosafety to address and halt the spread of genetically engineered organisms into the environment.²

Documentation: Text of 2014 NGO call to the Parties to the Convention on Biological Diversity and the Cartagena Protocol on Biosafety

² www.stop-the-spread-of-transgenes.org

Documentation: Text of 2014 NGO call to the Parties to the Convention on Biological Diversity and the Cartagena Protocol on Biosafe

Urgent request to address and halt the spread of genetically organisms into the environment!

Several examples of the uncontrolled spread of genetically engineered plants into wild populations and ecosystems have been documented: cotton in Mexico, oilseed rape in North America, Japan, Switzerland and Australia and grasses in the USA. There are also cases of repeated transgene presence in landraces or local varieties of crop plants such as maize in Mexico and rice in China. This increasing trend towards uncontrolled spread of transgenes into wild populations and ecosystems raises a high level of concern for the release of new organisms such as genetically engineered trees, fish and insects and the challenges posed by emerging applications of synthetic biology.

Genetic engineering and synthetic biology represent a radical break from widely recognized natural restrictions on genome regulation and interspecies genetic exchange. Maintaining the ability of organisms to develop under their evolved dispositions and naturally established restrictions and to participate in further evolutionary processes is a crucial aspect of the protection of biodiversity. In the same way that we seek to protect organisms and ecosystems from persistent chemical substances, we should also protect them from the uncontrolled spread of synthetic and genetically engineered organisms. In the short or long term, these organisms have a capacity to self-replicate, evolve and interact with other organisms in unpredictable ways and thereby represent a threat to ecological systems and their resilience.

There is a risk that we will not be able to recover the original biodiversity, as the dynamics of wild and cultivated native varieties will be altered. We cannot rely solely on gene banks, as they are able to preserve only a very small percentage of the genetic diversity present within centres of origin and of genetic diversity.

From a regulatory point of view, spatio-temporal control of genetically engineered organisms is necessary. It is a fundamental precondition for any risk assessment because no reliable predictions can be made concerning the consequences of artificially transformed organisms once they are released or escape into wider environments and become part of open-ended evolutionary processes.

Article 17 of the Cartagena Protocol on Biosafety requires Parties to prevent or minimize the risks of unintentional transboundary movements of genetically engineered organisms. The current trend of an increasing spread of these organisms outside of the managed settings for which they were approved threatens to enhance the potential for unintentional transboundary movements. Finally, the precautionary principle can only be implemented if genetically engineered organisms can be retrieved from the environment in case of emergency. This becomes impossible once transgenes move and accumulate in wild and landrace varieties.

→

While all genetically engineered organisms can pose risks to the environment and health, we particularly call for the prohibition of experimental releases, imports and commercialization of genetically engineered organisms if:

- a) they can persist and invade the environment if they escape their containment.
- b) they can not be withdrawn from the environment if this is required.
- c) it is already known that they can persist or have invasive behavior after release into the environment.
- d) their release may lead to transgene flow into populations of local varieties at centres of origin and of genetic diversity and the accumulation of transgenes in the genomes of native varieties.

Recently, the Convention on Biological Diversity via the Cartagena Protocol on Biosafety asked for information³

“on actual cases of unintentional transboundary movement and case studies related to their existing mechanisms for emergency measures in case of unintentional transboundary movements of living modified organisms that are likely to have significant adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, including information on existing rapid alert mechanisms and monitoring systems”.

The aim of this current report is to provide relevant information in this context and to give an update on factual, potential and future cases of the uncontrolled spread of genetically engineered organisms.

3 <https://www.cbd.int/doc/notifications/2015/ntf-2015-002-bs-en.pdf>

2. New evidence in documented cases of transgene escape

New evidence has emerged regarding previous case studies presented in publications (Bauer-Panskus et al., 2013 a,b). These are China (rice), Mexico (maize and cotton), Japan (oilseed rape), South Korea (maize and cotton) and Switzerland (oilseed rape).

2.1 Mexico: Genetically engineered maize and cotton



a) Previous knowledge: maize

Mexico is the centre of origin as well as a centre of diversity for maize (*Zea mays*). The presence of transgenic maize in Mexico was first revealed to the public through a study conducted by scientists from the University of Berkeley, who found transgenic constructs in traditional maize varieties in Sierra Juárez (federal state of Oaxaca) in the southeast of Mexico (Quist & Chapela, 2001). The findings were confirmed by other scientists (Serratos-Hernández et al., 2007; Piñeyro-Nelson et al., 2009; Dyer et al., 2009; Mercer and Wainwright, 2008). A NAFTA commission (CEC, 2004) concluded that maize imports from the US were responsible for the introgression of transgenic maize even in remote parts of Mexico. Maize was imported as food aid but was neither milled (to make it non-viable) nor labelled as being genetically engineered. In all probability, maize imported from the US was used as seed by Mexican farmers and thus found its way into traditional seed systems, which are based on seed exchange (Dyer et al., 2009). However, the real extent of the presence of transgenes in Mexican maize has never been adequately addressed.



b) Previous knowledge: cotton

Mexico is also one of the centres of origin for cotton species, which are the basis for nearly all commercial cotton varieties cultivated today. Wild cotton (*G. hirsutum*) populations are found mainly in the coastal regions of the southern states, and were recently described as an excellent source for widening the genetic base for breeding (Coppens d'Eeckenbrugge et al., 2014).

However, Wegier et al. (2011) were able to show that transgene constructs from genetically engineered cotton have spread into these wild cotton populations even though most of the commercially grown transgenic cotton is cultivated in northern and central Mexico, several hundred kilometres away from the regions where wild cotton is found. Every fourth sample proved positive for one or more transgenic constructs, and half of the populations were affected by transgenic introgression. In several samples, stacked constructs with up to four transgenes were found which are not present in any commercialised genetically engineered cotton event worldwide. Transports of unmilled cotton seed from the USA were thought to be a potential cause of the spread of transgenic cotton. US cotton seed is not milled before export to Mexico and thus remains fertile. Nevertheless, it is not yet fully understood how the transgenes introgressed.

c) New findings

In 2015, a report in a Mexican newspaper⁴ based on documents from several public agencies⁵, found that over the course of several years there has been extensive spillage throughout Mexico of transgenic maize and cotton. Between 2010 and 2013 alone, spillage of about 800 tons of transgenic maize and cotton was reported in Chihuahua, Guanajuato and Veracruz. All these cases involved accidents such as train derailments.

Table 1: Notified cases of spillage of transgenic maize and cotton in Mexico from 2010 to 2013 (Source: Contralinea)

Year	Federal State	Spillage (tons)	Crop	Company
2010	Veracruz	631	maize	CPIngredientes
2010	Guanajuato	20	maize	Almidones Mexicanos
2010	Guanajuato	20	maize	Almidones Mexicanos
2011	Chihuahua	86,32	cotton	Monsanto, Bayer
2012	Guanajuato	41,5	maize	Monsanto
2012	Chihuahua	4	cotton	Monsanto
2013	Chihuahua	?	cotton	?

In total, companies notified the spillage of 712.5 tons of genetically engineered maize kernels and 90.32 tons of genetically engineered cotton seeds.

According to the competent biosafety agencies, biosecurity measures, generally in the form of monitoring and destruction of volunteer plants, were undertaken to stop the escape of transgenic material. From the available information, the length of time of monitoring remains unclear, and it is also unclear whether the authorities were notified of all cases of unintended spillage through derailments. There is also no information available about earlier incidents of derailment and/or how these incidents were handled. In the absence of other information about the causes of transgene presence in Mexico, especially in cotton (see Wegier et al., 2011), transport losses such as the ones described above are a probable source.

4 <http://contralinea.info/archivo-revista/index.php/2015/01/18/trasnacionales-liberan-por-accidente-800-toneladas-de-transgenicos>

5 Comisión Intersecretarial de Bioseguridad de los Organismos Genéticamente Modificados (Cibiogem), instancia del Poder Ejecutivo federal integrada por los titulares de las secretarías de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (Sagarpa); Medio Ambiente y Recursos Naturales (Semarnat); Salud; Educación Pública; Hacienda y Crédito Público, y Economía, así como por el director general del Consejo Nacional de Ciencia y Tecnología (Conacyt).

2.2 South Korea: Genetically engineered maize, cotton and soybean

a) Previous knowledge

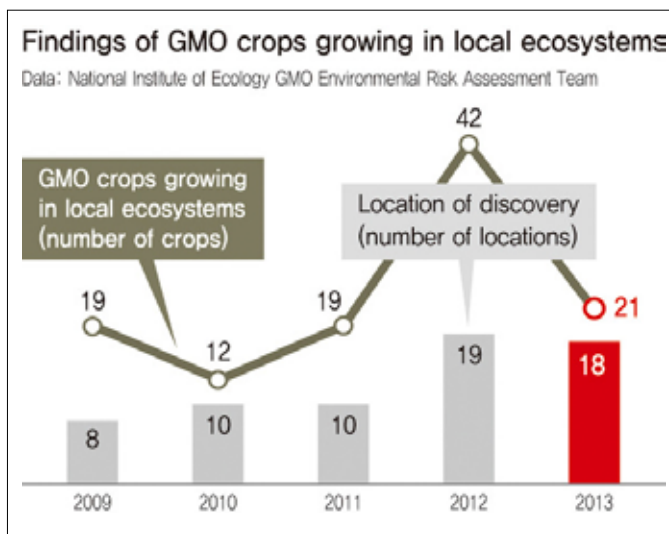
A news article from 2013 reported that the South Korean National Institute of Environmental Research (NIER) had tested plants in different parts of the country for the presence of transgenes (Jeong-su, K., 2013). In 2012, the institute took 626 samples of soybean, oilseed rape, maize and cotton from sites close to ports, factories where imported genetically engineered plants are processed as well as from farms and roadsides. 42 samples from 19 regions tested positive for the presence of transgenes. Maize was the most common plant with contaminations. According to the report, besides a few isolated plants, they found whole populations of transgenic plants close to the farms. Previous studies had shown the occurrence of genetically engineered plants growing in the vicinity of the harbours where they were imported (Lee et al., 2009; Park et al., 2010). Analyses found that some of these plants had undergone epigenetic changes which indicates unintended effects caused by the process of genetic engineering (Waminal et al., 2013).



b) New findings

According to the Korean press, further tests were conducted by the National Institute of Environmental Research (NIER) in 2013. Analysis showed that of 521 suspected samples from genetically engineered plants, 21 samples in eighteen regions proved positive.⁶

Most of the positive samples (nine) were found in areas near livestock farms. Genetically engineered plants were also found along import transportation routes (six) and near feed factories (three). The NIER report concluded that the discoveries are probably the result of corn and cotton imports and seeds that were spilled on the way to factories and farms.



It is unclear whether gene flow occurs to native populations and if any of those populations are persistent, or have become established because of permanent spillage. However, South Korea seems to be a primary example for the cultivation-independent establishment of genetically engineered plants in the environment, encompassing several species.

Figure 2: Genetically engineered crops in local ecosystems in South Korea from 2009 to 2013 (Source: hankyoreh, NIER)

⁶ http://english.hani.co.kr/arti/english_edition/e_national/675062.html

2.3 Switzerland: Genetically engineered oilseed rape



a) Previous knowledge

An empirical study on transportation losses of genetically engineered oilseed rape in Europe was conducted in Switzerland (Schoenenberger & D'Andrea, 2012). Samples proved positive for the presence of an enzyme that makes crops tolerant to herbicides with the active ingredient glyphosate. The high number of positive samples was remarkable because the import of transgenic oilseed rape has been prohibited in Switzerland since 2008. The findings lead to the conclusion that transgenic oilseed rape plants were able to survive along railway tracks for long periods because extensive glyphosate sprayings of these specific areas provided selective advantages. Another study (Hecht et al., 2013) confirmed these findings and identified loading terminals and stations as key risk areas.



b) New Findings

In a new publication (Schulze et al., 2014), feral genetically engineered oilseed crop plants (event GT73, Monsanto) were found along railway lines and in port areas in Switzerland. Feral glufosinate-resistant events MS8xRF3, MS8 and RF3 (Bayer) plants have been detected for the first time in Europe. Analyses showed outcrossing of GT73 oilseed rape into two non-genetically engineered oilseed rape plants. However, no outcrossing of transgenes into related wild species was observed by Schulze et al. (2014).

A recent follow-up study by Schulze et al. (2015) investigated the possible sources of herbicide tolerant oilseed rape along transportation routes in Switzerland. Analyses revealed that low level impurities of genetically engineered oilseed rape in imports of Canadian durum wheat is one source of feral genetically engineered oilseed rape in Switzerland. During the tests, the researchers found traces of oilseed rape events GT73, MS8xRF3, MS8 and RF3 in imports of durum wheat from Canada.

The reported results should be considered carefully as they may have far-reaching consequences for the regulation of genetically engineered crops.

- Firstly, the study found only traces of (on average 0,005%) genetically engineered oilseed rape in Canadian durum wheat, which is below the current limits of detection. According to the paper, the European Union Reference Laboratory for genetically engineered food and feed considers 0.1 % to be the lowest level at which results are satisfactorily reproducible between official laboratories. This means that contaminations in imports of food and feed will probably not be detected and spillage of oilseed rape seeds (or other transgenic seeds) into the environment might become a ubiquitous and permanent source of unintended presence of transgenic plants.
- Secondly, the results indicate, especially in the case of oilseed rape, that not only the crops where transgenic lines are on the market should be routinely checked for the presence of contamination from genetically engineered plants.

Research should also be conducted regarding the sources of the admixture in Canada, as there are various possible ways for oilseed rape to end up in imports of durum wheat. For example, there might be volunteer oilseed rape plants in wheat fields or contamination might also be due to an admixture in grain elevators.

c) New scientific evidence regarding the persistence of oilseed rape in the environment

From a biosafety point of view, transgenic oilseed rape poses a high risk of persistence in the environment. This is due to its biological features such as long outcrossing distances, gene flow through pollinators and wind, and long-term persistence of seeds in soil. Further, oilseed rape can hybridise with many other Brassica species, some of which are considered to be weeds. Gene flow from transgenic plants to weedy relatives is of special concern. According to Gressel (2015), “transgenic herbicide resistance poses a major risk if introgressed into weedy relatives.” Gene flow from oilseed rape to related species was recently discussed by Garnier et al. (2014) and Liu et al. (2013). Both studies highlight the aspect of uncertainty in the risk assessment of such events. According to Wang et al. (2013), EPSPS overexpression in oilseed crop plants may foster the fitness of glyphosate resistance in weeds leading to fitness advantages. Feral oilseed rape plants and their persistence in the environment were the subject of recent scientific studies. For example, Banks (2014) showed that feral oilseed rape in Scotland “can persist and flower outside the range of cropped oilseed rape plants. It has become part of the native weed and wildflower community, but to date has had no major ecological impact. The long term demographic changes in feral oilseed rape that were found in the 11 year study could not have been predicted from the initial early years when there were few populations or from prior estimates of risk carried out at small spatial scales.”

2.4 Japan: Genetically engineered oilseed rape



a) Previous knowledge

Although transgenic oilseed rape is not commercially cultivated in Japan, genetically engineered oilseed rape has been found growing there and 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 such as Kashima, Chiba, Nagoya and Kobe, as well as along transportation routes to industrial plants where oilseed rape is processed. Follow-up studies found ruderal populations along further transportation routes (Nishizawa et al., 2009) 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). Moreover, a publication by Mizuguti et al. (2011) came to the conclusion that oilseed rape populations are able to self-sustain over time. Undoubtedly, the percentage of transgenic oilseed rape in ruderal populations is constantly growing. In 2008, 90 percent of all plants tested in the proximity of Yokkaichi port proved to be genetically engineered. The first transgenic hybrid plants, a cross between *B. napus* and *B. rapa*, have been 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 therefore tolerant to the herbicides glyphosate and glufosinate.



b) New Findings

A study, conducted with the support of the Japanese authorities (Katsuta, 2015 et al), examined shifts in population distribution and occurrence of genetically engineered oilseed rape plants between 2006 and 2011 around the harbours. Genetically engineered *B. napus* was found around 10 of 12 ports, but its proportion in the populations varied greatly by year and location. While some populations were increasing or decreasing, no common trend could be detected. At some sites, the populations of genetically engineered plants were found to have been stable for several years even with no further imports. Contrary to other authors such as Schafer et al. (2011) and Aono et al. (2011), the authors did not find crosses between genetically engineered *B. napus* and its wild relative *B. rapa*. Further, no crosses were observed between *B. juncea* and *B. napus*. The authors concluded that the genetically engineered oilseed rape had so far not shown a pattern of invasiveness under the conditions in Japan.

The authors, however, did not assess findings from previous investigations indicating a changed biology in the plants. For example, according to previous research from Japan, the properties of feral herbicide resistant transgenic oilseed rape plants might have changed under the influence of climatic conditions and showed that some of the plants found were larger than normal. These plants have also become perennial (Kawata et al., 2009). This is a major change in the biology of the plants as oilseed rape and all other *Brassica* species cultivated in Japan are annual. Perennial forms of oilseed rape might have a significant impact on population dynamics. Perennial plants could have a higher probability of spreading their genes because they persist for a longer period. This could be seen as a factor supporting a higher fitness. The authors appeared unaware of publications showing that oilseed rape has become a part of the native weed and wildflower community in Scotland (Banks, 2014). This is in contradiction to some general assumptions made by Katsuta et al. regarding the biology of *B. napus*. Further, even though data were published, data collection had already stopped in 2011. Thus, more recent developments in persistence or invasiveness cannot be deduced from the publication.

2.5 China: Genetically engineered rice



a) Previous findings

Different transgenic lines have been detected in food imported from China since 2005, even though there is no commercial cultivation of genetically engineered rice in China. In most cases, these are the Bt rice lines Bt63 and KeFeng6. Bt63 was first tested in field trials around the year 2000, probably when scientists from a federal university in the Hubei region sold Bt63 to regional farmers without official authorisation (Greenpeace, 2005a,b). In 2011, German authorities found traces of non-approved transgenic rice in 16 of 561 samples of imported foodstuff from China (BVL, 2012). Interestingly, analysis of a register database of contamination reveals rice had the highest number of contamination incidents of all crops (accounting for a third of incidents) in the years 1997-2013, even though no genetically engineered rice was grown anywhere in the world (Price & Cotter 2015). Many of these incidents derive from cases of contamination of Bt63 rice from China.



b) New findings

The previous findings were substantiated by journalists and state inspections in 2014. According to the tests, genetically engineered rice was being sold in markets of two southern provinces (Hubei and Hunan). The allegation by the television network has been substantiated by the Beijing Entry-Exit Inspection and Quarantine Bureau after samples from the two provinces were tested positive for transgenic plants.⁷

c) New scientific findings on gene flow in rice

Several recent studies show that there are still many uncertainties regarding gene flow and persistence in rice. For example, a new publication on gene flow in rice showed that the distance of pollen flow might have until recently been underestimated. Field studies in China, Pu et al. (2014) concluded that other insects, in particular honey bees, are frequently attracted to rice plants and could have carried viable pollen over long distances, thus increasing the frequency of transgene flow. Several other publications investigated gene flow to weedy rice and showed considerable risk of transgene flow to weedy rice.

In regions where farm-saved rice seed is dominant, Serrat et al. (2013) found a high risk of genetically engineered red rice weed infestation that is increasing from year to year. Gene flow from herbicide resistant rice to weedy rice was also confirmed by Lu et al. (2014), who also demanded for measures to prevent the emergence of herbicide resistant weedy rice populations.

Finally, Yang et al. (2014) found that introgression of transgenes (Bt transgenes) into weedy rice can “significantly alter the segregation distortion pattern in hybrid progeny”. The authors concluded that transgenes with strong selection advantages (like Bt genes under high pest infestation) 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.

⁷ <http://globalvoicesonline.org/2014/07/28/chinas-state-media-reveals-unapproved-genetically-modified-rice-is-on-the-market/>
<http://www.scmp.com/news/china/article/1560737/genetically-modified-rice-found-sale-wuhan-supermarket>

3. Further transgene escapes and the lack of regulatory oversight

No regulation foreseen in several countries

There is already uncertainty about the present or future presence of certain genetically engineered organisms in countries like the USA. This is due to the fact that US GMO legislation has many loopholes and specific kinds of plants are exempted from regulation. For such plants, no risk assessment is required and the applicant does not have to publicly disclose whether or not they are conducting field trials or the genes they are using to confer the traits.

The loopholes in the US regulatory system were revealed in 2010 when Scotts convinced USDA to declare that another variety of Kentucky bluegrass engineered for glyphosate tolerance should not be subject to the agency's oversight (Waltz, 2011). The US Department of Agriculture (USDA) requires regulation of transgenic plants only if "plant pests" – genetic material from bacteria and viruses – were used in the transformation process.⁸

In recent years, USDA has granted unregulated approval of several transgenic plants. Camacho et al. (2014) and Ledford (2013) have provided an overview of plants that have escaped regulation in the US. This lack of regulatory oversight also means that nothing is known about whether these plants have undergone field trials or are already on the market.

3.1 Transgene trees: Pine trees (USA)



In January 2015, it became known that the US Department of Agriculture will not regulate a genetically engineered tree line developed by US company, ArborGen. The loblolly pine trees (*Pinus taeda*) are engineered with genetic material from another pine species (*Pinus radiata*), *E. coli*, bacteria *Liquidambar styraciflua* and *Arabidopsis thaliana*.⁹ From what is known about ArboGen's project, the loblolly pines are engineered to have denser wood.

The pine trees may now be planted in field tests and commercialised without regulatory oversight or environmental risk assessment. Loblolly pine is an economically important and common native tree in the southeast of United States. Gene flow to natural forests will be inevitable if they are commercialised. This scenario is underpinned by scientific publications such as those by Williams et al. (2006), who showed that the probability of long-distance dispersal of transgenic conifer seeds at distances exceeding 1 km is close to 100%. Pine trees are known for long distance pollination over many kilometers.



8 <http://www.centerforfoodsafety.org/press-releases/3713/new-genetically-engineered-tree-to-avoid-federal-oversight-completely>

9 http://www.aphis.usda.gov/wps/portal/?1dmy&curile=wcm:path:/aphis_content_library/sa_our_focus/sa_biotechnology/sa_regulations/ct_reg_loi

3.2 Herbicide tolerant grasses (USA)



Apart from ArborGen's pine trees, there are other cases of genetically engineered plants that are exempted from regulation in the US, according to documents released and published on the agency's "Regulated Letters of Inquiry" web page.¹⁰ For example, Scotts Miracle-Gro developed several grass events that USDA decided did not fall under its oversight:

- a tall fescue (*Festuca arundinacea*) variety engineered to grow shorter, thicker and darker green than its conventional counterparts. The genetic material comes from various undisclosed plants and is integrated using particle bombardment. According to the company, the grass will require less mowing and fewer nutrient inputs and is also glyphosate tolerant (Waltz, 2015);
- varieties of St. Augustine grass (*Stenotaphrum secundatum*) and Kentucky bluegrass (*Poa pratensis*) engineered to be shorter, thicker, darker green and resistant to glyphosate.



According to Waltz (2015), Kentucky bluegrass and St. Augustine grass are currently undergoing field trials, and tall fescue is in greenhouse development.

Scientific evidence indicates that escape of transgenic grass varieties seems almost inevitable. Kentucky blue grass is an invasive species with weedy characteristics. A recent study describes the invasion process and shows that Kentucky blue grass has become the dominant species in some prairies in the Northern Great Plains and North and South Dakota (DeKeyser et al., 2015).

3.3 Herbicide tolerant alfalfa (USA)



Herbicide tolerant alfalfa (*Medicago sativa*) which is resistant to herbicides with glyphosate as the active ingredient was commercialised in the US in 2011 and planted on about 750,000 hectares in 2013, according to the industry lobby group ISAAA.¹¹ Although it is widely accepted that alfalfa is a high risk plant in terms of weediness and persistence in the environment, there is still no empirical study investigating transgene escape from commercialised herbicide tolerant alfalfa in the US. Results from ongoing investigations for the evaluation of landscape-level effects of transgenic alfalfa led by the US Department of Agriculture are not available yet.¹²



¹⁰ http://www.aphis.usda.gov/wps/portal/?1dmy&urile=wcm:path:/aphis_content_library/sa_our_focus/sa_biotechnology/sa_regulations/ct_reg_loi

¹¹ www.isaaa.org/resources/publications/biotech_crop_annual_update/download/biotech-crop-annual-update-alfalfa.pdf

¹² www.ars.usda.gov/research/projects/projects.htm?accn_no=424252

However, previous studies indicate that the biology of alfalfa may contribute to it becoming increasingly feral. For example, Bagavathiannan & Van Acker (2009) showed

“high genetic diversity, perenniality, quick regrowth potential, persistence, symbiotic nitrogen fixation, deep tap root system, drought and cold tolerance, and seed dormancy. With these traits, alfalfa is equipped to invade and dominate unmanaged habitats. Feral alfalfa populations can and will act as bridges for long-distance gene flow and facilitate the adventitious presence of novel traits in the environment.”

Therefore, it remains an open question whether and how the uncontrolled spread of transgenic alfalfa into the environment can be stopped.

Regarding possible gene flow to wild relatives, it is widely assumed that in North America, no sexually compatible wild relatives of alfalfa exist. However, according to Canadian risk assessors, in other regions of the world such as Europe, Asia, the Middle East, and North Africa, native populations of various relatives of *Medicago sativa*, as well as other perennial *Medicago* species, are present and introgression of genes from alfalfa to wild relatives is possible.¹³

3.4 White maize (Philippines)



Genetically engineered maize that is resistant to herbicides and produces Bt proteins has been grown in the Philippines since 2002. Many farmers in the Philippines use open pollinated varieties for traditional corn production, which means that the transgenes can persist in regional varieties as soon as gene flow is established. In fact, in an investigation carried out by Greenpeace, many traditional varieties of white maize were found to be contaminated with genetically engineered plants (Greenpeace, 2013).



White corn is one of the most important food crops in the Philippines. After rice, it is the staple food of 20% of the population, primarily in the southern regions of the Visayas and Mindanao. Analysis of samples purchased in a market in Mindanao showed the presence of two events of genetically engineered corn. The levels of contamination found were substantial, with percentages of contamination up to 40%.

One reason for this large scale contamination is the failure of national authorities to properly register the genetically engineered varieties being commercialised. Further, regulation is insufficient to allow effective protection of regional landraces. For this reason, Greenpeace is also warning that the cultivation of genetically engineered so-called “Golden Rice” in the Philippines might lead to large scale contamination of the regional varieties of rice. The Philippines is a known centre of diversity for rice.

13 www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/medicago-sativa-l-/eng/1330981151254/1330981232360#b7

3.5 Maize (South Africa)



The persistence of transgenes in the informal seed system in South Africa has recently been detected and described. South Africa is another example of a tradition where recycling and sharing of seeds is common, and may contribute to the spread and persistence of transgenes in maize on a local or regional level.

Genetically engineered maize was introduced into South Africa in 1997. A study by Iversen et al. (2014) analysed transgenes in maize plants (leaves) and seed batches collected in a village where insect resistant maize was previously promoted and grown. Transgenic material was found in only one of the 796 leaf samples. However, genes for insect resistance (from MON810) were found in two (10%) of the seed batches, indicating that the MON810 event may have become part of the locally recycled maize. According to the study, in both cases farmers were unaware of the presence of the transgenes. The authors concluded

“that transgenes are mixed into seed storages of small-scale farming communities where recycling and sharing of seeds are common, i.e. spread beyond the control of the formal seed system.”

The authors found that the conditions in South Africa, which are „typical“ for a country in the global south with a high percentage of small scale farming, management of transgenic plants is highly challenging because

“... fields of different farmers are close together, informal practices of seed recycling and sharing are more common, several different varieties of maize are commonly planted together, and the small fields make it comparatively more inconvenient to include buffer zones between non-GM and GM crops.”

Because of the small sample size, the significance of this study is limited and results have to be confirmed in larger scale studies. However, the results comply with models of transgene dispersal and persistence in smallholder agriculture that were published, for example, by Aheto et al. (2011) who found that genetically engineered crops could remain in cultivation even if their admission has expired or has been retracted. Further, Aheto et al. (2013) concluded that

“from an ecological perspective, introduction of GM crops would lead to uncontrolled large-scale spread and persistence of transgenes within the small-scale agricultural systems in Africa with unpredictable recombination and evolution in crop meta-population.”



3.6 Genetically engineered animals: Mosquitoes (Brazil & Panama)



Oxitec male OX513A genetically engineered mosquitoes are intended to mate with wild females and produce offspring which die as larvae. Releases of many millions of genetically engineered males are intended, over time, to reduce the total population of mosquitoes. The mosquitoes released in the experiments are *Aedes aegypti*, which transmit the tropical disease dengue fever. Oxitec's strain of the *Aedes aegypti* mosquito is genetically engineered to die at the larval stage in the absence of the antibiotic tetracycline, which acts as a chemical switch to allow breeding in the laboratory.

However, there are several mechanisms which could enable the mosquitoes to survive in the environment:¹⁴ In the laboratory, 3% of Oxitec mosquito offspring survive in the absence of tetracycline (Phuc et al., 2007). When genetically engineered mosquitoes were fed cat food containing industrially farmed chicken, which contains the antibiotic tetracycline, the survival rate increased to 15-18%. Oxitec originally hid this information¹⁵ but later admitted to an 18% survival rate of larvae fed on cat food in a published paper (Massonnet-Bruneel et al., 2013) A number of studies have found that *Aedes aegypti* mosquitoes can breed in septic tanks where there can be high levels of contamination with antibiotics such as tetracycline.¹⁶ *Ae. aegypti* also commonly live in areas where discarded takeaways are likely to contain meat contaminated with tetracycline. Oxitec uses a diet supplemented with 30 µg/ml of tetracycline to breed its mosquitoes in the lab. The tetracycline derivatives oxytetracycline (OTC) and doxycycline (DOX, used to prevent malaria) could also allow the genetically engineered mosquitoes to breed. Oxytetracycline can be found at concentrations above 500 µg/g in animal manure and doxycycline at up to 78516.1 µg/kg dry weight in broiler manure, which is likely to be more than enough to inactivate the killing mechanism.¹⁷ The issue of genetically engineered mosquitoes breeding in areas contaminated with tetracycline has not been considered in risk assessments in either Panama or Brazil. The percentage of surviving genetically engineered mosquitoes could increase if resistance to the genetic killing mechanism evolves over time (Alphey et al., 2011). Increased survival rates would reduce the effectiveness of any population suppression effect over time and potentially allow the genetically engineered mosquitoes to establish in the wild.

Oxitec releases of genetically engineered mosquitoes in Brazil started in 2011 and in Panama in 2014. Brazil's regulator has also approved commercial releases of genetically engineered mosquitoes, even though no results from the trials have been published as yet. Lack of oversight was knowingly caused by breaching the requirements of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD): Neither Brazil nor Panama has submitted any information to the Biosafety Clearing House in relation to genetically modified mosquitoes.



14 The following information is taken from documents provided by GeneWatch UK, an NGO based in Great Britain (GeneWatch UK, 2014).

15 GeneWatch, Friends of the Earth, Third World Network PR: Company conceals evidence that genetically modified mosquitoes may have high survival rate in wild (12th January 2012)
[www.genewatch.org/article.shtml?als\[cid\]=492860&als\[itemid\]=569476](http://www.genewatch.org/article.shtml?als[cid]=492860&als[itemid]=569476)

16 for References see GeneWatch UK, 2014

17 for References see GeneWatch UK, 2014

4. Future cases and new methods in genetic engineering

The following section of the report outlines some possible future cases. The examples include eggplant in India and salmon in North America as well as genetically engineered flies that might be released in the Mediterranean region in Europe. These examples are far from comprehensive. As already mentioned, lack of oversight leads to a high level of uncertainty, which will undoubtedly be relevant to future cases. For example, genetically engineered plants such as “Golden Rice”, Bt poplar in China (for review see Then & Hamberger, 2010) may become highly relevant for future gene flow from transgenic plants into the environment. Also organisms used for energy production such as specific grasses or algae, can become wide-ranging possible future cases. (Then et al., 2010).

Further, new methods of genetic engineering not only make it possible to change the genome even more radically, they can also change the pattern of heredity and enhance gene flow into native populations.

4.1 Bt eggplant (India and Bangladesh)



Eggplant (*Solanum melongena*), also known as brinjal or aubergine, was domesticated in South-East Asia from a wild relative *Solanum incanum* (known as wild brinjal). Its primary centre of diversity is situated in the Indo-Burma region where primitive cultivars and weedy forms exist. Diversification led to the accumulation of diversity in several secondary centres of diversity (Hurtado et al. 2015).

In India, Bt eggplant was approved for commercialisation in 2009. Commercialisation was stopped following public opposition, and a moratorium was declared. However, field trials were allowed in 2014.¹⁸ As India is a centre of diversity for this vegetable species, there is concern that gene flow will lead to the presence of transgenes in traditional cultivars and wild or weedy relatives. Whereas investigations of the potential for pollen-mediated, crop-to-wild gene flow are scant, a recent study confirmed that hybridisation is possible between cultivated and wild brinjal in southern India (Davidar et al. 2015) and crop transgenes could spread to wild brinjal populations that occur nearby.

Another study (Mutegei et al., 2015) underpinned the importance of wild eggplant species for future breeding efforts. According to the authors, eggplant “ranks high among crops whose wild gene pools are underrepresented in ex situ collections and warrant urgent conservation.”

Even though the cultivation of Bt eggplant is not allowed in India, there is widespread suspicion that Bt eggplant seed from Bangladesh is being smuggled into the state of Bengal and cultivated by farmers.¹⁹ In Bangladesh, cultivation of Bt eggplant (Event EE1, trade name BARI Bt Begun-1, -2, -3 and -4) was approved in 2013. Bangladesh is also considered as a centre of origin for eggplant. The crop’s centre of diversity reaches throughout Southeast Asia and as far as the Philippines, where field trials of Bt eggplant were started around 2010 (Samuels, 2012).



18 www.hindustantimes.com/india-news/govt-allows-field-trials-for-gm-mustard-brinjal/article1-1279197.aspx

19 www.business-standard.com/article/news-ians/commercial-bt-brinjal-seeds-infiltrating-bengal-from-bangladesh-114110501181_1.html

4.2 Genetically engineered trees: Eucalyptus (USA / Brazil)



There are two important cases of releases of genetically engineered eucalyptus trees that should be considered in this report.

In Brazil, in April 2015, the responsible authority, the Brazilian National Technical Commission on Biosafety (CTN-Bio), approved the commercial use of a transgenic eucalyptus event produced by the company FuturaGene (Anonymous, 2015).²⁰ The trees are engineered to produce higher yields.

The decision was heavily criticised, partly because in 2008, the CBD had stated (decision IX/5 (t) of the UN CBD Conference of the Parties from 2008)²¹ that a precautionary approach should be taken in the case of transgenic trees. So from the perspective of CBD, this example is of special importance as the CTNBio ignored the UN CBD decision regarding genetically engineered trees and stated:

*“Trans-boundary movement of transgenic eucalyptus plantlets or seeds is highly improbable and accidental seed propagation elsewhere is even more improbable. ... Therefore the release of this GM tree is solely a Brazilian question and no other country or group of countries has the right to interfere in our decision.”*²²

In contrast to Brazil, the US authorities have not yet approved the commercialisation of genetically engineered eucalyptus trees. Large-scale field trials have, however, been ongoing in some southern US states for several years now. ArborGen developed the so-called Freeze Tolerant Eucalyptus (FTE) and filed for deregulation of the product in 2013.

*“FTE lines [...] were developed by the introduction of the Crepeat Binding Factor (CBF2) gene from Arabidopsis into a fast growing but freeze susceptible commercial hybrid genotype of E. grandis x E. urophylla. The potential for reduced growth by overexpression of CBF genes in the FTE lines has been significantly mitigated by the use of a cold-inducible promoter that limits the expression of the CBF gene under conditions where this would not be desirable. In addition to the CBF2 gene, these FTE lines contain a selectable marker used extensively in plant transformation and a gene expression cassette that prevents pollen development. This pollen control cassette provides an additional level of confinement by restricting gene flow from the FTE lines.”*²³

The long distance dispersal and persistence of eucalyptus is limited by the fact that the species are not usually frost tolerant. However, in this case, the eucalyptus trees were provided with a clear fitness advantage. With the development of freeze-tolerant eucalyptus, the cultivation range is likely to further expand in the southeast of the US and to new areas of the Pacific Northwest.²⁴



20 <http://labiotech.eu/futuragenes-genetically-modified-eucalyptus-approved-in-brazil/>

21 <https://www.cbd.int/decision/cop/?id=11648>

22 <http://stopgetrees.org/wednesday-email-from-ctnbio-member-paulo-paes-de-andrade/>

23 <http://www.noticeandcomment.com/Petition-for-Determination-of-Non-regulated-Status-for-Freeze-Tolerant-Hybrid-Eucalyptus-Lines-fn-21240.aspx>

24 Centre for Food Safety: Genetically Engineered Trees: The New Frontier of Biotechnology. <http://www.centerfor->

The trees are genetically engineered to inherit genetic material intended to limit uncontrolled spread into the environment. However, given the life span of the trees, it is unclear if these built-in mechanisms will be safe enough in the long-term and, for example, continue to ensure functional genetic stability in all trees.

Biology of eucalyptus

Eucalyptus is a genus of trees that belongs to the *Myrtaceae* family. There are around 500 species, most of them endemic to Australia. Eucalyptus is the most common tree in plantations around the world and is cultivated in regions such as North and South America, Europe, Africa, the Middle East, China and India. The most important species comprise *Eucalyptus grandis*, *E. urophylla*, *E. Pellita*, *E. globulus*, *E. nitens*, *E. dunni*, *E. camaldulensis*, *E. tereticornis* and *E. saligna*, as well as several hybrids like *E. urophylla* x *E. grandis*.

Many species are closely related and can exchange genetic material. Long-distance dispersal over several kilometres has been reported (examples discussed in OECD, 2014). For example, natural F1 hybrids between *E. regnans* and *E. macrorhyncha* were observed and reported by Ashton and Sandiford (1988) in forests located over 5 km away from the closest stands of *E. regnans*. Eucalyptus species are mainly pollinated by birds, insects and mammals, which contributes to long distance gene dispersal. The principal pollinators of eucalyptus are bees, flies and beetles (OECD, 2014).

The potential of eucalyptus to escape from plantations and persist in the environment or even invade territories has been discussed frequently. The Invasive Plant Atlas of the United States lists six eucalyptus species as invasive of natural areas, including (Invasive Plant Atlas):²⁵

<i>Tasmanian bluegum</i>	<i>Eucalyptus globulus</i> Labill.
<i>redbox</i>	<i>Eucalyptus polyanthemus</i> Schauer
<i>swampmahogany</i>	<i>Eucalyptus robusta</i> Sm.
<i>black peppermint</i>	<i>Eucalyptus salicifolia</i> (Sol.) Cav.
<i>forest redgum</i>	<i>Eucalyptus tereticornis</i> Sm.

One study in Brazil, specifically designed to examine the abilities of *E. grandis* and *E. grandis* x *E. urophylla* to invade and establish in areas of native vegetation lying adjacent to plantations, demonstrated no risk for these trees (da Silva et al., 2011). However, recent scientific studies also showed results relevant for risk assessment that indicated a high level of uncertainty in regard to the risks of transgenic trees escaping into wild populations:

- Catry et al. (2015) showed massive dominance of *Eucalyptus globulus* in Portugal. Eucalyptus wildlings were found in 60 % of samples and across all natural regions.
- Costa e Silva et al. (2014) showed that long distance gene flow between *Eucalyptus globulus* populations in Tasmania led to heterosis effects in progeny. The authors concluded that heterosis derived from long-distance pollinations may be sufficient to counter local mal-adaptation, at least in the first generation.

foodsafety.org/reports/2637/genetically-engineered-trees-the-new-frontier-of-biotechnology

- For the US, “models to predict the likelihood of invasiveness have produced conflicting results“ (Lorentz and Minogue, 2014).
- For Florida, data from Lorentz and Minogue (2015) indicate that under specific favourable conditions, eucalyptus seedlings may establish within or proximate to planted stands. According to this publication, *E. grandis* and *E. camaldulensis* are already part of the regional biodiversity in some areas of Florida and show a high potential of invasiveness.

4.3 Genetically engineered animals: Salmon (Canada/Panama)



Growth hormone (GH) genetically engineered Atlantic salmon (*Salmo salar*) developed by AquaBounty Technologies is one of the first transgenic animals being considered for commercial use (AquAdvantage salmon). This genetically engineered salmon variety is close to the commercialisation stage in North America.

It was engineered with a growth hormone-regulating gene from a Pacific Chinook salmon and a promoter from an ocean pout. These genes enable it to grow year-round instead of only during spring and summer. The purpose of the modifications is to increase the speed at which the fish grows, without affecting its ultimate size. The main concern regarding the approval of this genetically engineered salmon is that it will escape to the wild and seriously damage wild salmon populations and whole ecosystems.



According to AquaBounty, the company will put several safety measures in place that they say will prevent the escape of transgenic fish. The company claims it will only produce sterile female fish. Further, AquaBounty claims that it will only produce its fish in a scheme involving: growing the eggs at its Prince Edward Island facility in Canada, flying them to a land-based facility in Panama to be raised, and finally shipping the processed salmon to U.S. markets. However, in spite of AquaBounty’s claim that they will only produce sterile female fish, the US Food and Drug Administration risk assessment presented data indicating that up to 5% of the fish could be fertile and able to reproduce.²⁶

A 2011 study published by Canadian scientists concluded that if genetically engineered Atlantic salmon were to escape they could succeed in breeding and passing their genes into the wild (Moreau et al., 2011). Also a recent investigation by Sundström et al. (2014) found that transgenic salmon would have a significant effect on ecosystems. According to the researchers, the transgenic fish would significantly reduce the survival and growth of the populations that were invaded if they were first allowed to grow larger under hatchery conditions before being released.

²⁶ U.S. Food and Drug Administration Center for Veterinary Medicine. “Draft Environmental Assessment: AquAdvantage Salmon.” May 4, 2012 (published December 26, 2012), page 86.

4.4 Genetically engineered animals: Olive flies (Europe)



In August 2013 and in July 2015, it was made public that the UK company Oxitec was planning to release genetically engineered olive flies (*Bactrocera oleae*) in Spain (Catalonia) in an experimental field trial.²⁷ The Oxitec flies are manipulated with synthetic DNA, which is a mix of maritime organisms, bacteria, viruses and other insects. The Oxitec flies are genetically engineered so that any female offspring are programmed to die at the larval stage, while the male offspring survive and continue to mate. As a result, it is thought that the population of native olive flies (that can cause economic damage in olive production) will decrease; it may even become extinct. Similar organisms were established with Mediterranean fruit flies (Medfly), Diamondback moth and other species.²⁸



Fig. 2: Olive tree in organic agriculture in Greece with trap for olive flies (Foto: Testbiotech)

If the technology goes ahead as planned by Oxitec, it could, after a long period of time, lead to a substantial reduction of the whole fly species population in the areas affected. Biodiversity could be severely disrupted, with all the various potential consequences and side-effects for the sensitive balance of the ecosystem, the environment and food production systems. Additionally, there is a high likelihood that the artificial genes will end up being permanently present in the native populations.

²⁷ http://gmoinfo.jrc.ec.europa.eu/gmo_report.aspx?CurNot=B/ES/15/06

²⁸ www.oxitec.com/agriculture/our-solution/

Olive flies are regarded as a species that spreads rapidly in a suitable habitat. Over time their offspring might be distributed throughout the Mediterranean region and all the locations where native populations occur. The flies might spontaneously cross boundaries and come into contact with a wide range of environmental conditions, the artificial genes might be expressed in various genetic backgrounds (there are genetic differences in the regional varieties of the flies) and be exposed to environmental stressors such as climate change. While Oxitec claims their strains were genetically stable in the laboratory, nobody can predict genetic stability or the ecological behaviour of these insects once released.

The negative socioeconomic and also potential transboundary impacts would be, for example, that organic farmers might not be able to avoid their products coming into contact with these flies, and therefore their food products might even contain the biotech-larvae. Markets could be lost as a consequence – and in an extreme scenario, the whole of the harvest in the Mediterranean area could be affected if consumers reject those products.

Not only do these genetically engineered insects pose environmental risks, they also raise ethical questions: Should we for economic reasons allow the release of organisms that are genetically engineered to possibly cause the extinction of a whole species?

4.5 Synthetic Biology and Synthetic gene technologies

New methods in synthetic gene technology (genome editing) such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) in combination with DNA synthesis, are speeding up the creation of an increasing number of new organisms inheriting synthetic DNA. At the same time, these techniques can be used to change the genome far more radically than previously possible. In the near future, more and more genetically engineered or synthetic organisms might be released into the environment in ever shorter periods of time. This would inherently increase the likelihood of gene flow from those organisms to the environment.

Nucleases are proteins (enzymes) which can be used to splice DNA, hence the term “DNA scissors” or “gene scissors”. These tools were discovered some time ago, but were only able to be used to “cut” DNA in relatively few places. In recent years, several new nucleases have been developed that in principle allow for targeted DNA introduction or modification at any chosen site in the genome. The current star of the nuclease family is known as CRISPR-Cas. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and consists of a guide-RNA region, which can match with a targeted DNA sequence. RNA is capable of mirroring and ‘recognising’ DNA structure, so that the CRISPR-Cas system can be directed to specific sequences in the genome.

The Cas enzyme, which is coupled with the tracer-RNA, operates as the 'DNA scissor' and can 'cut' a single DNA strand or both simultaneously. Mutations often occur as the cell's own mechanisms seek to repair the breaks, causing, for example, genes to be silenced. CRISPR-Cas also allows synthesised DNA to be introduced to the site. The Cas enzyme can also be applied to silence genes without cutting the DNA. The system is surprisingly simple and efficient to operate. Since the possibilities of the CRISPR-Cas system were first discovered some two or three years ago, publications have grown rapidly and there are already commercial applications for its use in laboratory animals. Other gene scissor systems such as TALEN (Transcription Activator-Like Effector Nucleases) and Zinc Finger Nucleases function along similar lines but have proved more difficult to operate. Despite the fact that multiple uses for DNA scissors have been identified, there is as yet no understanding of how they actually function in detail.

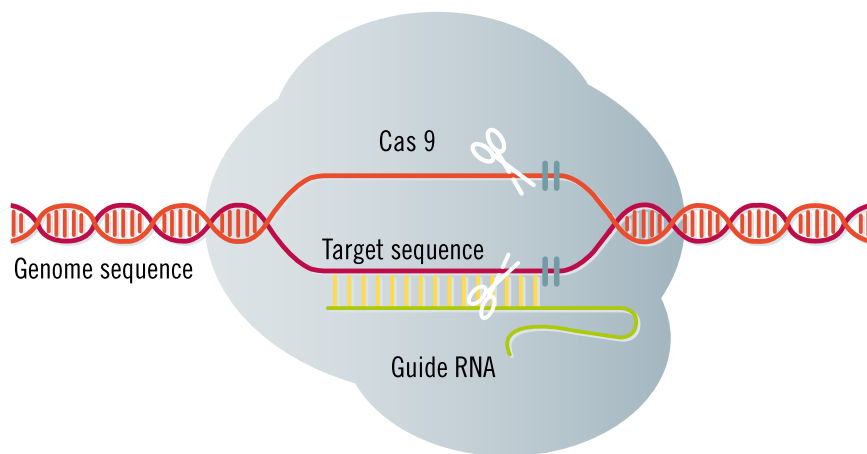


Fig. 3: CRISPR / Cas: The nuclease CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) can be tailored to bind to specific DNA sequence that is identified via a guide RNA. It is combined with a protein (enzyme) that can cut the DNA to silence genes or to introduce additional synthetic DNA.

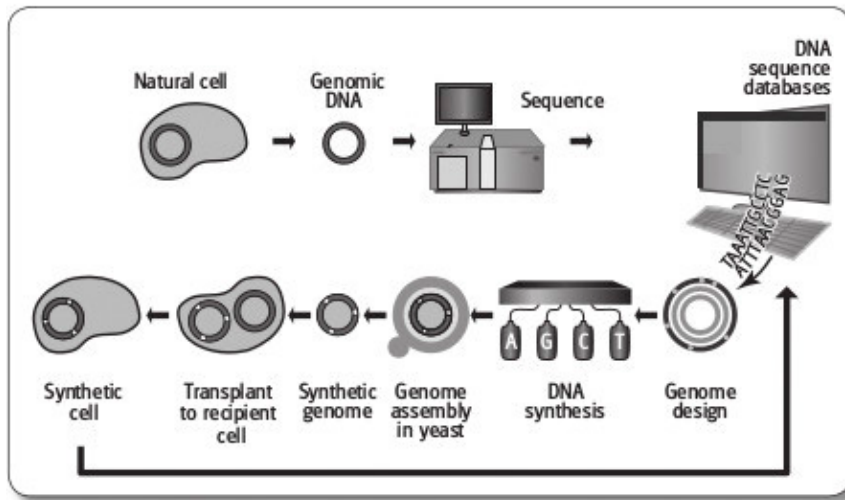


Fig 4.: DNA analysis and DNA synthesis go hand in hand. Source: US Presidential Commission for the Study of Bioethical Issues, (redrafted in “Handbuch Agro-Gentechnik“, C. Then, Oekom Verlag 2015).

Some of the organisms will have new genetic conditions or biological characteristics that cannot be compared with those from existing biodiversity. And in the same way, new organisms may appear unchanged in their genetic condition even though their genome has been partially or completely replaced by synthetic DNA. In 2010, a microorganism was presented whose DNA was completely re-synthesised in the laboratory (Gibson et al., 2010). In 2014, a publication showed that a whole chromosome in yeast had been replaced by synthetic DNA (Annaluru, et al., 2014).

Further, we have to be aware that Synthetic Biology can not only interfere with the structure of the genome, but also with the pattern of heredity. So-called gene-drives are used to change the pattern of heredity in order to spread artificial DNA more rapidly into native populations (Gantz & Bier, 2015). Usually, a genetic change in one organism takes a long time to spread through an entire population. With a gene drive nearly all the offspring will inherit the change. As shown in recent articles, gene drives were successfully established in flies by mutagenic chain reaction (MCR) that enables that DNA inserted by CRISPR on one chromosome to copy itself to its partner in every generation. This means that the newly introduced DNA will speed through a population exponentially faster than normal (‘gene drive’). Synthetic DNA engineered into a fly could therefore spread through a large population within a relatively short period of time (see Gantz & Bier, 2015; Ledford, 2015).

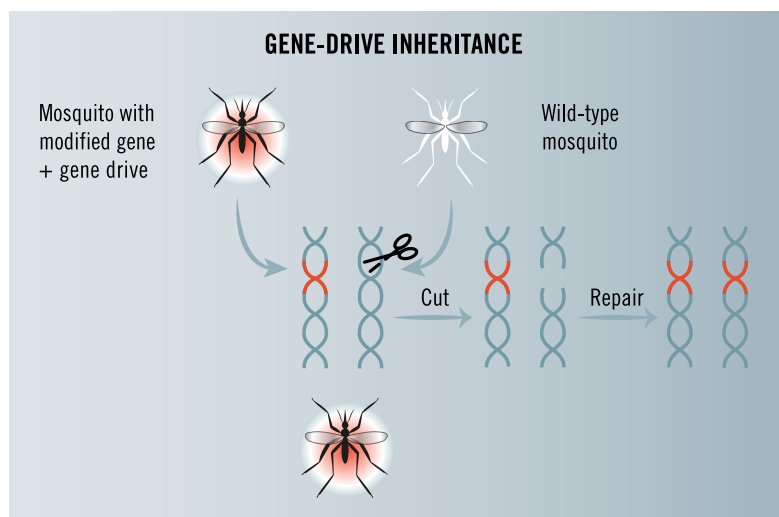


Fig. 5: Gene drive such as mutagenic chain reaction is based on synthetic gene technology and use of CRISPR enabling a change in the structure of DNA on one chromosome to copy itself to its partner chromosome in every generation. This means that the newly introduced DNA will speed through a population exponentially faster than normal. (adapted from Ledford 2015)

These experiments raised a fiercely controversial debate amongst scientists (Ledford, 2015). Even the scientists who published the experiment (Gantz & Bier 2015) raised questions about potential hazards of future applications. At least to current knowledge, the predictability of any release of such organisms that can act as gene drives seems to be very low. Any risk assessment would necessarily suffer from a substantial amount of non-reducible non-knowledge (see Boeschen et al, 2006) On the other hand, if negative impacts occur, there are no sufficiently reliable risk management strategies available to stop a gene drive organism once it has been released and capable of interacting with native populations.

This triggers new challenges for international regulation. Currently, there is no internationally binding regulation that could be used to enforce a global restriction or ban on the release of such organisms. This issue was also addressed in the Nature editorial of 6 August 2015.²⁹

“What is new is the advent of CRISPR. This adds an extra dimension to the debate, because it makes gene drives much easier to create and could dramatically accelerate the timeline for a potential release — accidental or intentional. Researchers and funding agencies should take note, and efforts to understand the ecological consequences of a gene drive should be made an urgent priority. Regulators and the wider world need to keep pace with the rapid development of CRISPR technology, and there is little time to waste.”

29 www.nature.com/news/driving-test-1.18118?WT.ec_id=NATURE-20150806&spMailingID=49256879&spUserID=MjA1NTM1NDgzNQS2&spJobID=740835078&spReportId=NzQwODM1MDc4So

5. Discussion

This report adds to the existing evidence of the uncontrolled spread of genetically engineered organisms. Taking into account existing cases (Bauer-Panskus et al 2013 a, b), we already have several species (such as cotton, grasses, maize, oilseed rape and rice) that are affected by transgene flow into native populations or traditional seed systems in centres of crop diversity, where it is leading to the cultivation-independent establishment of transgenes in the environment.

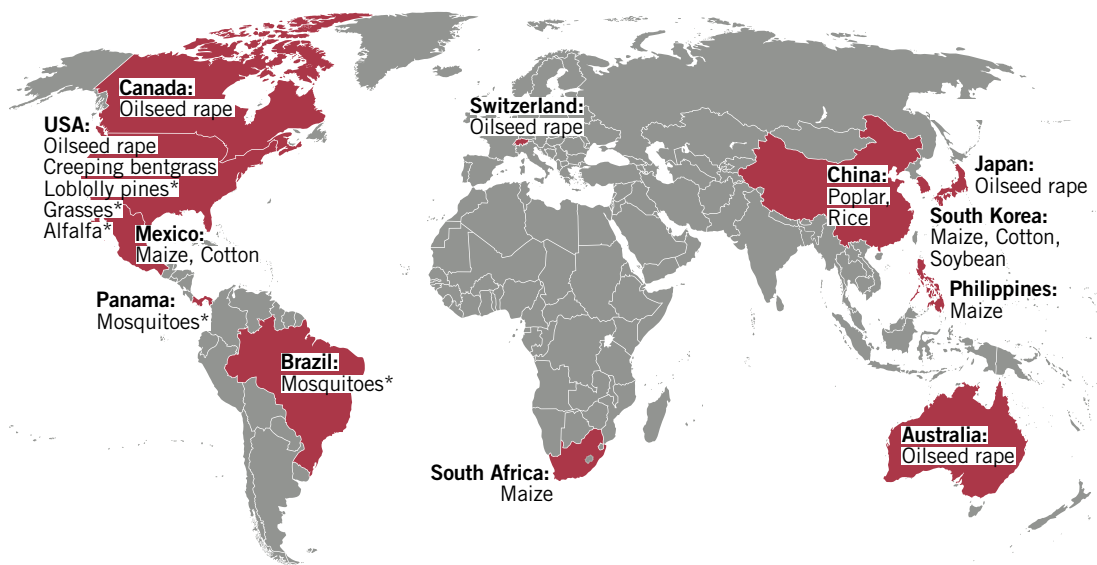


Figure 6: Overview of case studies of uncontrolled spread of genetically engineered organisms (* = lack of regulatory oversight or missing scientific data)

Further, there are cases of plants that are currently being grown, or could be grown, that have been identified as potential sources of transgene escape. As there is a substantial lack of oversight and monitoring in this context, gene flow may actually occur without being noticed.

Potential future cases include some genetically engineered animals such as salmon and insects. Finally, new methods in synthetic gene technologies, so-called gene drives can be applied in a way that will change the pattern of heredity and foster the establishment of synthetic DNA in native populations.

This list of cases is not comprehensive: For example, in organisms used for energy production such as grasses or algae, there are wide-ranging possible future cases.

5.1 What can we learn from existing cases?

Some of the cases do have the potential for spontaneous spread and also for unintentional transboundary movement. So what kind of risk do they pose for biodiversity? In general, the overall long-term impact on ecosystems is hard to predict or cannot be predicted at all. In this regard, transgenic plants can be compared to alien species. Even if the biological characteristics of a species are known, its potential to persist or invade under new environmental conditions very often still cannot be predicted (BfN, 2005).

In comparison there are major differences between the spread of genetically engineered organisms and the invasive potential of an alien species. In the case of genetic engineering, one must consider the spread of technically inserted genetic information within the gene pool of a species that has already adapted to its environment. The dynamics of proliferation within established species and the resulting impacts can be very different from the pattern of the ecological potential of an alien species within a new environment.

In the context of genetic engineering, specific attention should be given to the genetic stability and functionality of the inserted DNA. Unlike alien species, genetically engineered crops contain technical DNA constructs, very often 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 influence of climate change or in their interaction with other stress factors, this can have unexpected effects in the crops that may also bring new risks for the environment or health risks if they are used for food.

Relevant risks are the proliferation of plants pests (if genetically engineered plants or their crossings with wild relatives become more susceptible), unwanted effects in the plants composition (if for example environmental stress results in genetic instabilities caused by the additional DNA) or higher invasiveness than predicted. If our most relevant food plants are impacted it could result in a tremendous hazard for food security.

In cases of uncontrolled spread in the environment, risk assessment would require taking evolutionary dimensions into account. Evolutionary processes make it possible to turn events with a low probability of ever happening into events that may feasibly happen (Breckling, 2013). For example, outcrossing into wild species could be enhanced by climate or other environmental change. There are cases published showing that especially hybrids of cultivated species with wild species develop a higher fitness under stress. Consequently, it is not possible to predict the long-term ecological impact of genetically engineered organisms that escape spatio-temporal control.

Some of these questions were addressed in the international call already mentioned for the Member States of the CBD to take measures against spontaneous unintentional transboundary movement of genetically engineered organisms (www.stop-the-spread-of-transgenes.org/). This call identified the need for regulatory action:

“From a regulatory point of view, spatio-temporal control of genetically engineered organisms is necessary. It is a fundamental precondition for any risk assessment because no reliable predictions can be made concerning the consequences of artificially transformed organisms once they are released or escape into wider environments and become part of open-ended evolutionary processes.

(...) The precautionary principle can only be implemented if genetically engineered organisms can be retrieved from the environment in case of emergency. This becomes impossible once transgenes move and accumulate in wild and landrace varieties”

5.2 New risks require new regulation

The increasing potential of synthetic biology and synthetic gene technologies (genome editing) makes the need for regulatory action more pressing than ever. In regard to the protection of biodiversity, this implies that the number of different technically derived organisms being released into the environment within the next few years could increase dramatically. Therefore, the likelihood of negative consequences will also increase - not only stochastically.

It is undoubtedly difficult to perform risk assessment on an organism that shows, for example, more complex changes in its genome. Its biological properties might be hard to compare with existing organisms. We have to face the fact that in general we are not able to assess the risks of these organisms in a way that we can reliably conclude upon long-term safety, especially if there is no spatio-temporal control. However, we are not aware of any of any internationally binding framework that could be used to impose a global ban on the release of organisms derived from genetic engineering or synthetic biology if spatio-temporal control cannot be established. But such international regulation is urgently required: For example, in the case of a fly inheriting a gene drive, most existing regulatory frameworks would require risk assessment to be carried out, and then, based on available evidence and/or plausibility, would come to decisions that might diverge from country to country – while the gene drive flies could just simply move across boundaries.

6. Conclusion and demands

There is no doubt that in the age of the “Anthropocene”, biodiversity has already been severely impacted by human activities such as those in agriculture and land use. Gene flow from transgenic organisms and/or from organisms derived from synthetic gene technologies, is adding an extra level of risk to biodiversity.

The only choice we have in dealing with these problems is to strengthen the precautionary principle. In the same way that we seek to protect ecosystems from long-lasting chemical substances that can accumulate in the environment, we should seek to protect the ecological and evolutionary integrity (Pimentel et al., 2000) of natural biodiversity by preventing the uncontrolled spread of synthetic and genetically engineered organisms in the environment.

However, current international regulatory frameworks and instruments are not sufficiently robust to effectively prevent gene flow from genetically engineered organisms to native populations. Consequently, we need much stronger regulatory tools to allow a global ban on the release of any synthetic organisms (or transgenic organisms) that cannot be controlled in their spatio-temporal dimension, especially if they have a long-term potential for spontaneous unintentional transboundary movements.

So we see an obvious pressing need for international and national political action. Starting with the recommendations made by Bauer-Panskus et al. (2013a,b), we propose establishing international regulations that will make it possible to ban any release of genetically engineered or synthetic organisms if

- they can persist and invade the environment if they unintentionally escape their containment;
- there are major doubts about whether they can be withdrawn from the environment within a reasonable period of time if this is so required in cases of urgency;
- it is already known that they will persist or show invasive behaviour after release into the environment.

Further, all possible measures should be taken to prevent the transgenes which have already escaped from spreading even further. In addition, the cultivation of genetically engineered plants in regions where they can contaminate farmers’ seed saving systems should be stopped as quickly as possible to prevent farmers from unintentionally promoting the spread of the transgenes within the agro-ecological systems.

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