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Transgene escape -
Global atlas of uncontrolled spread of
genetically engineered plants

A Testbiotech report by
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Content

Summary

This "transgene escape" report documents several case studies where genetically engineered plants have spread uncontrolled into the environment. In some instances from North and Middle America, we can assume that transgenes from species such as bentgrass, oilseed rape and cotton have already escaped permanently into the environment or wild populations. In other cases such as maize (corn), rice and poplar there is a high likelihood that this will happen in the near future.

Apart from commercial cultivation and experimental field trials, there are further cases showing that the uncontrolled dispersal of genetically engineered plants can be due to the import of raw materials and transport of viable grains for food and feed production.

Several factors can facilitate the spread of transgenic plants. Besides some plants such as grasses with a naturally high potential for persistence and invasiveness, one further major factor in the uncontrolled spread of transgenes are wild relatives, which can cross with the crop plants where they are grown.

It is difficult to make reliable predictions which of the genetically engineered plants will persist or become invasive, and what their long-term environmental impact will be. The authorisation of transgenic plants with increased fitness to withstand changing climatic conditions will add to the uncertainty.

Currently there is insufficient research into the causes and consequences of the uncontrolled spread of transgenic plants.

In some countries there appears to be a negative correlation between political support for the commercial growing of genetically engineered plants and a failure to research the risks of an uncontrolled spread of transgenes into the environment.

This report makes several recommendations: Most importantly, measures should be put in place immediately to stop any further uncontrolled spread of genetically engineered plants into the environment as far as possible. 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.

1. Introduction

According to Nature magazine, genetically engineered plants were grown on 170 Million hectares globally in 2012 (Gilbert, 2013). The plants were mostly soybean, oilseed rape, maize (corn) and cotton. Nearly all of them had two technical traits: herbicide tolerance and / or production of insecticidal proteins.

The persistence and invasiveness of genetically engineered plants in the environment was one of the most heatedly debated issues when the technology was first introduced (see for example Tomiuk et al., 1996). Later in 2001, broader public awareness of the issues was triggered after scientists from the University of Berkeley found genetically engineered maize in remote regions of southern Mexico (Quist & Chapela, 2001), even though there were no field trials in Middle America at this time.

It is a well-accepted fact that genetically engineered plants can move beyond the field sites and cross with wild relatives (Marvier & Van Acker, 2005). But there is to date – apart from individual publications (Ellstrand, 2012) - no systematic coverage in literature of documented cases. There is, furthermore, hardly any development of risk assessment related to the long- term introgression of transgenes into natural (or nearly natural) eco-systems or agricultural systems.

The focus of this report is on the identification of cases of spontaneous proliferation of genetically engineered plants in natural or agricultural environments from where it is unlikely that they can be retrieved. Especially in those cases when transgenes have moved into populations of wild relatives, they are likely to have escaped permanently from effective control.

In parallel to this report, Testbiotech commissioned a legal dossier to examine needs and options for regulation to stop the uncontrolled spread of genetically engineered organisms (Krämer, 2013). The evidence presented in this dossier, points to a pressing need for stricter regulations on a global scale.

2. Some general background on the spread of genetically engineered plants

This section of the report contains an overview of factors and mechanisms that influence the potential spread of (genetically engineered) plants.

Invasive potential of agricultural plants

Domesticated plants used in agricultural production have often lost some of their potential for persistence and invasiveness, and frequently need cultivation by farmers to survive. Normally a high degree of domestication will mean lower potential to survive under natural conditions.

However, some 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 with periods in between of "de-domestication", or reversion to a wild form (Vigueira et al., 2013). The potential for spread is also highly relevant in the context of agrofuels, where grass species with a high degree of invasiveness such as miscantus (*Miscanthus × giganteus)* and switchgrass (*Panicum virgatum L.*) are cultivated (see for example Ecker et al., 2013; Lewis 2013). In the case of sorghum (*Sorghum sp.*) which has wild weedy relatives within the group of Poaceae this is also an issue that needs to be considered (Arriola & Ellstrand,N. C., 1996; Adugna & Bekele, 2013). Finally, genetically engineered rice such as the so-called Golden rice, which will be grown in regions where weedy rice populations exist, is likely to show a substantial potential for geneflow and persistence as well as invasion of other rice fields (Lu & Yang 2009, Chen et al., 2004).

There are also examples, where cultivated plants or hybrids between wild and cultivated plants have remarkably shown a higher degree of fitness than that of wild relatives and have even displayed invasive qualities. Wild coconut has, for instance, been completely replaced by cultivated varieties (Ellstrand, 2003). Another important example in the US is a spontaneous hybrid between wild radish (*Raphanus raphanistrum*) and cultivated (*Rapahanus sativus*) radish, which developed invasive hybrids. The so-called California wild radish shows a higher degree of fitness than the two parental species (Ridley & Ellstrand, 2009) and has displaced the original species completely within a few life cycles (Hedge et al., 2006). Spontaneous hybridisation between sugar beet and the wild sea beet (*Beta vulgaris* subsp. *Maritima)* in Europe has led to the evolution of the noxious weed beet that has resulted in over a billion dollars of losses to the European sugar industry (Ellstrand 2003).

Plants with a life cycle of more than one year such as trees, or in the case of crop plants, alfalfa, have a high potential for moving into wild populations and becoming persistent (Bagavathiannan et al., 2011, 2012). Genetically engineered alfalfa is authorised in the US for commercial growing[1] and genetically engineered poplar have been grown in China for more than ten years (see for example Wang, 2004; Lu & Hu, 2011).

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1   http://www.monsanto.com/newsviews/Pages/roundup-ready-alfalfa-supreme-court.aspx

Gene flow to wild relatives

Centres of origin are regions with a high genetic diversity. These are the regions where our agricultural plants originate.

The wild species from which our agricultural plants originate are an important genetic resource for further plant breeding if, for example, there is a requirement for improved resistance to climate change or plant pests. Future plant breeding could be jeopardised if transgenes spread into these resources. Similarly, agriculture in the centres of origin could be permanently damaged if transgenes spread into regional landraces.
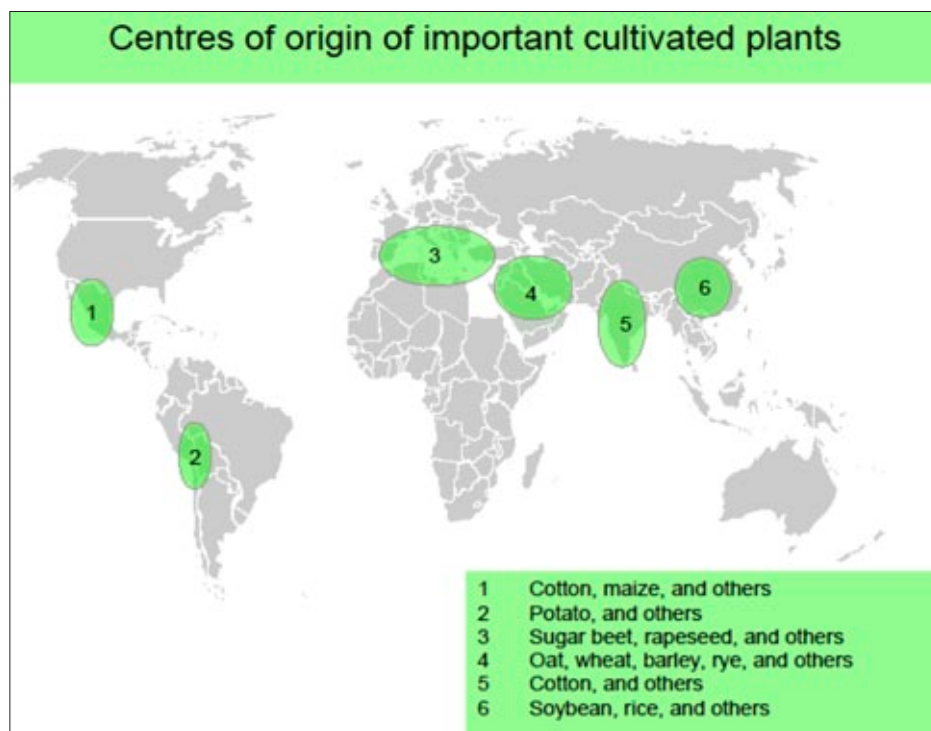
Figure 1: Centres of origin of important cultivated plants

Several factors foster the gene flow, persistence and proliferation of transgenes in these regions. Over time, the plants growing here have adapted to the environmental conditions and can cross with wild relatives. Further, in several such regions, there are established informal systems for seed exchange that can lead to unnoticed transferrals of contaminated seeds. Moreover, in many instances, the necessary organisational, legal and financial requirements to control and monitor the spread of genetically engineered plants effectively in these regions have not been met .

There are several examples showing that genetically engineered plants have already arrived
in the centres of origin:

> Bt maize (corn) in Mexico

> Bt and herbicide tolerant cotton in Mexico

> ❯ Bt rice in China
> ❯ herbicide tolerant oilseed rape in Europe
> ❯ Bt poplar in China

In general, the probability of transgenes spreading uncontrolled into the environment is greatly increased if gene flow occurs into related non-domesticated species. Wild original species and wild relatives with which they can cross exist – at least at a regional level and not just in the centres of diversity – for all relevant cultivated plants. We found the following cases, which verify outcrossings into wild relatives:

Zapiola & Mallory-Smith (2012) found transgenic hybrids which were identified as crosses of wild rabbitfoot gras (*Polypogon monspeliensis*) with transgenic bentgrass (*Agrostis stolonifera*) ;
transgenic cotton was identified in Mexico in 2012 in populations of wild cotton (Wegier et al., 2012); hybrids between transgenic oilseed rape (*B. napus)* and *B. rapa* were identified in Japan (Aono et al., 2011); crosses between transgenic oilseed rape *(Brassic napus)* and *Brassica rapa* were identified in Canada (Warwick et al., 2007; Simard et al., 2006)

The impact of climate change

According to many experts, ongoing climate change will lead to substantial changes in the communities of wild species. In particular, plants and animals with invasive potential will spread massively. Clements & Ditommaso (2011) expect climate change to cause an exponential growth in the populations of invasive plant species. Parepa, Fischer & Bossdorf (2013) show that increasing variability in environmental conditions (such as a concentration of nutrients in the soil), can enhance invasion of alien plant species. There has already been a global increase in the frequency of extreme conditions such flooding or severe drought due to climate change.

This in turn leads to new uncertainties regarding the invasiveness of genetically engineered plants. Those plants engineered to have traits such as drought resistance might acquire a higher fitness in a changing climate. There is also the possibility that genetic instability could occur in the plants that was not observed under normal climate conditions, which might lead to higher fitness in transgenic crops.

For example a publication from the Netherlands has concluded that when temperatures are higher there is a higher risk of overwintering and volunteers in potatoes and sugar beet (van de Wiel et al., 2011). This can also enhance the potential of transgenic plants to persist.

3. Properties of transgenic plants and their impact on persistence and invasiveness

In many cases, the fitness of transgenic plants is dependent on specific environmental conditions.

Herbicide tolerant plants

Genetically engineered herbicide tolerance is mostly considered to be a neutral trait having no effect on yield or fitness when introduced into conventional plants. Even where several herbicide tolerance genes were introduced into oilseed rape, this did not cause lower fitness compared to the parental conventionally bred plants (Simard et al., 2005). However, higher fitness has been observed where this trait has outcrossed to crop weeds or cultivated plants that can become weedy e.g. sugar beet, lucerne and oilseed rape. Oilseed rape in particular has a large number of weedy relatives.

Londo et al. (2010, 2011) show that drift of glyphosate leads to enhanced selection advantages in herbicide tolerant oilseed rape (if for example they are growing close to the fields) and fitness advantages in weeds that cross with transgenic oilseed rape. Wang et al. (2013) show that weedy rice, which has been crossed with a herbicide resistant rice can have higher fitness even when it is not treated with the supplementary herbicide. The respective hybrids were larger and produced more seeds.

Bt plants

The enhanced fitness of plants producing insecticidal Bt-toxins is also under discussion. There are many studies which show a fitness advantage for plants producing Bt toxins. For example, Bt oilseed rape showed a higher fitness in experiments where the plants were under pest insect pressure (Mason et al., 2003). There were similar findings for rice (Zhang et al. 2012).

Several further studies examining the effects of crossing genetically engineered plants with wild relatives concluded that Bt genes conferred higher fitness. Snow et al. (2003) were the first to demonstrate higher fitness in such hybrids in their study on Bt sunflower. Under controlled conditions, Bt-poplar hybrids (*Populus tremula×Populus tremuloides*) affected by pest insects showed a higher fitness compared to their wild type (Hjältén et al., 2012).

Interestingly, *Brassica rapa* plants that were crossed with Bt oilseed rape produced 1.4 times more seed than the wild type (Vacher et al., 2004). Wild relatives such as *Brassica rapa* and *Raphanus sativus* are likely to achieve advantages by crossing with Bt plants (Letourneau & Hacker, 2012). Further, according to a model created by Meier et al. (2013), a higher fitness can be expected if Bt-genes are outcrossed into wild *Raphanus raphanistrum* plants because these will show a higher rate of survival if they are attacked by pest insects.

Stress resistance and other genetically engineered plants

The development of genetically engineered plants able to perform better under stress conditions such as drought or higher salt concentrations have been on the agenda of industry for several years. Some products are already in the pipeline. For example, a new variety of maize (corn) with enhanced

drought resistance produced by the US company Monsanto is now being grown in the US[2]. Also in the US, cold resistant eucalyptus trees are in the process of being commercialised[3].

Such traits will enhance the fitness of the genetically engineered plants and if geneflow occurs also enhance the fitness of wild relatives (Mercer et al., 2007; Beckie et al., 2010). There are some other characteristics of genetically engineered plants that can lead to higher fitness: According to Claessen et al. (2005), a change in the composition of fatty acids (such as a higher content in stearic or lauric fatty acid) causes higher fitness in oilseed rape.

4. Case studies

In this chapter, we have listed several cases where there has already been an uncontrolled spread of transgenes into the environment, and cases where there is a high probability that this will happen in the near future.

4.1 Case study: Genetically engineered Creeping bentgrass *(Agrostis stolonifera)*

Country: USA

Plant: Creeping bentgrass (*Agrostis stolonifera*)

Mode of escape: Hybridisation with wild growing bentgrass, hybridisation with wild relatives

Region: Oregon, Idaho

Trait of escaped transgenes: Herbicide tolerance

Gene: cp4epsp

Description:

In 2002, Scotts started field trials with genetically engineered creeping bentgrass (*Agrostis stolonifera*) in central Oregon (US) near the city of Madras. The field trial covered an area of 160 ha. In 2004, it was found that pollen from transgenic bentgrass (which had been made tolerant to herbicides containing glyphosate as the active ingredient) had intro-gressed into wild growing bentgrass populations. Outcrossing

Figure 2

into wild bentgrass populations and the sexually compatible species *Agrostis gigantea* took place at distances of up to 14 kilometres; cross-pollination by using trap plants was even found at a distance of 21 kilometres (Watrud et al., 2004). In 2003, a storm caused the wide scale dispersal of transgenic bentgrass pollen. Attempts by Scotts to remove all genetically engineered plants failed. In 2007, the USDA fined Scotts 500.000 US $ for failing to comply with regulations.[4]

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2   http://monsanto.mediaroom.com/2013-06-17-Monsantos-Drought-Tolerance-Trait-In-Genuity-DroughtGard-Hybrids-Receives-Final-Major-Import-Approval-From-China

3   https://federalregister.gov/a/2013-04519

4   http://www.usda.gov/wps/portal/usda/usdahome?contentidonly=true&contentid=2007/11/0350.xml

During the years that followed, transgenic bentgrass was found all over the investigated area (Zapiola et al., 2008).

Tests conducted along irrigation canals in 2006 showed that more than half of the investigated plants contained the glyphosate tolerance gene cp4epsps (Reichmann et al., 2006). Dispersal is mediated through outcrossing as well as seed dispersal. Currently, transgenic bentgrass can be found in irrigation canals, roadside ditches, on grassland and in fields. Genetically engineered bentgrass also escaped from a second field trial in Idaho (Mallory-Smith, 2011). Several years after the end of the field trial, transgenic plants were found in Canyon County (Idaho) as well as Malheur County in nearby Oregon. Apparently, transports were the reason for the dispersal.

According to a recent publication, even interspecific hybridisation with related species takes place. Hybrids were identified as rabbitfoot grass (*Polypogon monspeliensis*) that had introgressed into feral transgenic bentgrass (Zapiola & Mallory-Smith, 2012). Bentgrass is a perennial plant with many compatible related species. Several relatives have invasive traits and are considered to be weeds. Field trials in the 1990s have already shown that herbicide tolerance genes can introgress into related species like *A. canina*, A. *capillaris* or *A. castellana* (Wipff & Fricker, 2001).

A. stolonifera is wind-pollinated and produces large amounts of pollen. Up to 100.000 pollen per square meter were detected at a 2-3 kilometre distance from a bentgrass field. Pollen has low weight and remains fertile for a long time. An additional problem in the US bentgrass case is the fact that Oregon is the main production area for bentgrass seeds. Bentgrass seed from Oregon is also exported to the EU and other regions.

Figure 3 (Zapiola & Mallory-Smith 2012, with kind permission): Comparison of creeping bentgrass (left), transgenic intergeneric hybrid *x Agropogon littoralis* (centre), and rabbitfoot grass panicles (right).
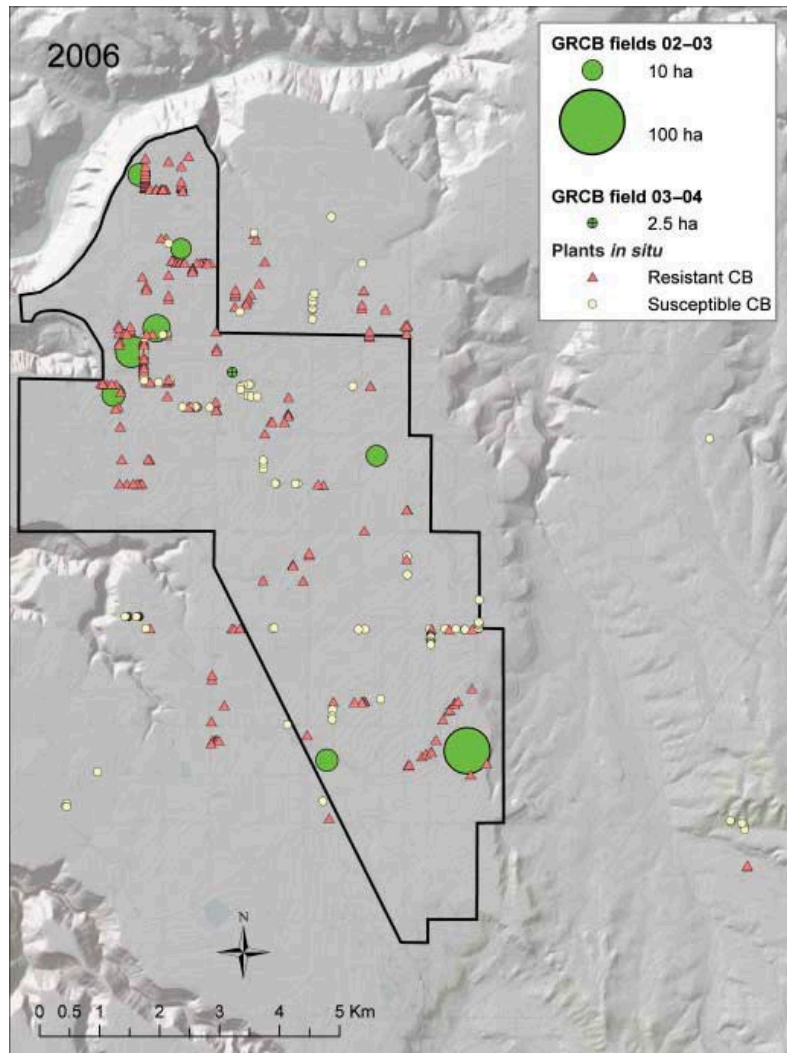
Figure 4 (Zapiola, et al., 2008, with kind permission): Creeping bentgrass (CB) plants tested in situ in 2006. Location of transgenic glyphosate-resistant CB (GRCB) fields in 2003 and 2004 (fields to scale but not true to shape), and glyphosate-resistant and susceptible CB plants found in situ (a single point may represent more than one plant). Black line, limit of GRCB control area.

Biological profile: Creeping bentgrass *(Agrostis stolonifera L.)*

Family: *Agrostis,* True grasses (*Poaceae*)

Centre of origin/diversity:
Creeping bentgrass originates from Europe, Asia and Africa.

Cultivated species:
Cultivated varieties are used as feed, in lawn mixtures, particularly for golf courses.

Major producing countries:
Creeping bentgrass is common in the northern hemisphere from Europe to Western Asia and in North America (distribution in the US see http://plants. usda.gov/java/profile?symbol=AGST2) as well as in the southern hemisphere.

Spread of pollen: By wind (Zapiola & Mallory-Smith, 2012)

Spread of seeds: Water, animals, transport; apart from seeds, the main mode of dispersal is by stolons.

Farthest pollen-mediated outcrossing distance measured to date:
21 km (Watrud et al., 2004)

Seed persistence/dormancy:
Up to four years (Zapiola et al., 2008)

Hybridisation potential with other crop plants:

❯ Common bent (*A. capillaris L.*), which is used in lawn mixtures and as forage grass, common bent is listed as an invasive species
http://www.issg.org/database/species/ecology.asp?si=1365&fr=1&sts=sss&lang=EN

❯ brown or velvet bent (*A. canina L.*), used in lawn mixtures

Both species are mainly cultivated in north eastern parts of the US.

Creeping bentgrass is part of a species complex, which are able to hybridise with each other (Rotter et al., 2010). According to Wipff (2002), some of the hybrids are so common that they have specific names. Examples are:

❯ *A. x murbeckii Fouillade (A. stolonifera x A. capillaris);*

❯ *A. x bjoerkmanii Widén (A. capillaris x A. gigantea).*

Hybridisation with wild relatives:

❯ *Agrostis* species (Wipff, 2002):

❯ *A. castellana*

❯ *A. gigantea*

❯ *A. mertensii Trinius*

❯ *A. vinealis Schreber*

Also *Polygonon* species belong to the complex of species which are compatible with *Agrostis* (Wipff, 2002). Examples are

❯ Rabbitfoot grass (*Polypogon monspeliensis*) (Zapiola & Mallory-Smith ,2012); hybrids are so common that they have their own names (*x Agropogon littoralis*);

❯ *Polypogon viridis (Gouan) Breistr.*, hybrids are called x *Agropogon robinsonii*

❯ *P. fugax*

Weediness / invasiveness:

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).

Wild relatives with potential for invasiveness/weediness:

A. canina, A. capillaris, A. castellana, A. stolonifera, A. gigantea, A. vinealis (Wipff, 2002). Common bent (*A. capillaris)* is listed as an invasive species http://www.issg.org/database/species/ecology.asp?si=1365&fr=1&sts=sss&lang=EN

Possible transgene-mediated fitness advantage:

Application of complementary herbicide confers advantage. .

4.2 Case study: Genetically engineered cotton in Mexico

Country: Mexico

Plant: Cotton (*Gossypium hirsutum)*

Mode of escape: Hybridisation with wild cotton species (*Gossypium hirsutum)*

Region: Pacific and gulf coast in southern Mexico

Trait of escaped transgenes: Herbicide tolerance, insect toxicity

Gene: cry1Ab/c, cry2A, cp4epsps, pat/bar

Description:

Mexico is one of two centres of origin from which are all cotton varieties cultivated today originate. Wild cotton (*Gossypium hirsutum*) populations are found mainly in the coastal regions of the southern parts of Mexico. Based on genetic differences, eight metapopulations can be distinguished. In a pilot study published in 2011 (Wegier et al., 2011), were able to show that transgene constructs from genetically engineered cotton have spread into these populations. Genetically engineered cotton has been planted in Mexico since 1996.

Figure 5

According to industry sources, 161.500 ha of herbicide tolerant and Bt cotton varieties were planted in 2011. Almost all commercially grown cotton is cultivated in northern Mexico, several hundred kilometres away from the regions where wild cotton grows. Nevertheless, every fourth sample proved positive for one or more transgenic constructs, and half of the populations were affected by the contamination.

In many - but not all - cases, the contaminated spots were located in relative geographical proximity of regions where genetically engineered cotton is grown. There were also positive samples in locations more than 750 kilometres away from transgenic cotton fields.

Several samples contained stacked constructs with up to four transgenes. Stacked events such as the ones found in the wild are not present in any commercialised genetically engineered cotton event worldwide. Therefore, it can be assumed that several individual transgenes have hybridised with each other over time. A similar observation was published in regard to herbicide tolerant oilseed rape in Canada (Simard et al., 2005). This would mean that transgene constructs spread within the wild cotton populations.

As cotton is mainly autogamous, outcrossing via pollen is relatively rare, but the findings of Wegier et al show that crossing did take place. A main cause for the spread of transgenic cotton is probably the transport of cotton seeds from the US, to be used as feed. US cotton seed is not milled before export to Mexico and thus remains fertile. Nevertheless, it is not fully understood how transgenes introgressed and questions remain that require detailed investigation if we are to preserve the integrity of wild cotton populations.
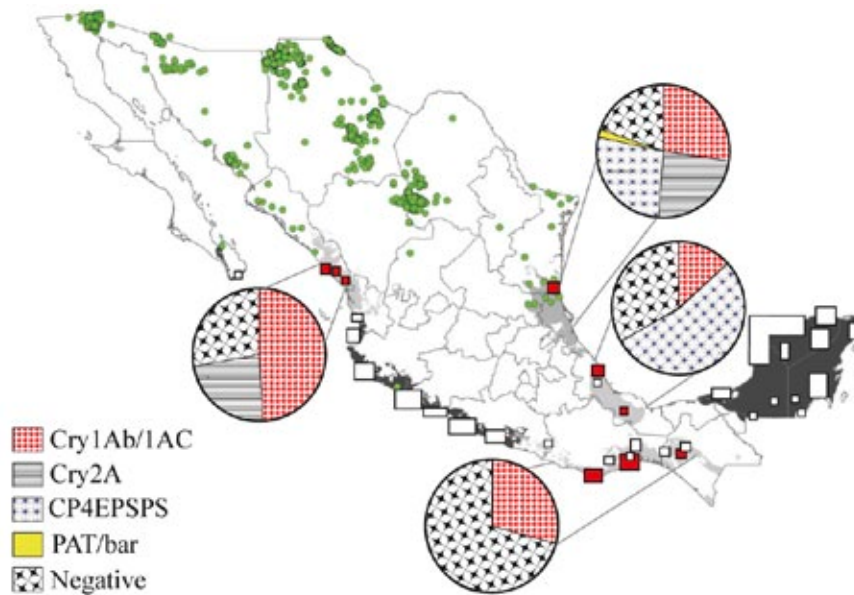
Figure 6 (Wegier et al., 2011, with kind permission): Map of Mexico showing the regions where GM cotton cultivars have been approved for planting, as well as wild cotton metapopulations and populations positive for recombinant protein presence. GM cotton cultivation sites are plotted as green circles; metapopulations without recombinant proteins are coloured in dark grey; metapopulations with recombinant proteins are in pale grey; wild cotton populations with transgene presence are plotted as red squares while populations without transgenes are depicted as white squares. Pie charts with the frequency of particular recombinant proteins are set aside each transgene-harbouring metapopulation.

Biological profile: Cotton *(Gossypium hirsutum)*

Family: Mallows (*Malvaceae*)

Centre of origin:
Africa, Arabian peninsula, Central America (Mexico), Australia. Diploid species
(*G. herbaceum* and *G. arboreum*) are old world species, whereas teraploid species originate from
Central America (*G. hirsutum)* or South America (*G. barbadense)*.

Cultivated species:
G. hirsutum, G. barbadense, G. herbaceum, G. arboreum

Major producing countries:
China, India, USA (OECD, 2008), Pakistan, Russia, Republic of Kazakhstan .

Spread of pollen:
Mainly self-pollination, to a lesser extent pollen is also spread by wind or insects such as bees or
bumblebees (OECD, 2008; Heuberger et al., 2010; Llewellyn et al., 2007). Under natural condi-
tions, 7.5% pollen was still fertile after 32 hours (Richards et al., 2005).

Spread of seeds:
In natural conditions (i.e. in wild cotton species), seeds are spread by water, birds or mammals.
Cultivated cottons seeds are mainly spread by transport, or by farm animals and their waste. A
small percentage of the seeds is still fertile after passing through the digestive system of farm ani-
mals such as cows (Eastick & Hearnden, 2002).

Farthest pollen-mediated outcrossing distance measured to date:
1600 meters (Van Deynze et al., 2005)

Seed persistence/dormancy:
Wild cotton seeds can be fertile for 30 years or more in field conditions (Wegier, 2012).

In cultivated varieties, seed dormancy was practically eliminated in the course of domestication.
(OGTR, 2008).

Hybridisation potential with other crop plants:
G. hirsutum und *G. barbadense* are sexually compatible (Shilla et al., 2013), the introgression of *G.
barbadense* in *G. hirsutum* is more likely than the other way round (OECD, 2008).

Hybridisation with wild relatives:
G. hirsutum is compatible with tetraploid species *G. mustelinum*, *G. darwinii*, and *G. tomentosum.*
Wild cotton (*G. hirsutum)* can be found in Mexico and southern parts of USA (EPA, 2006).

Weediness / invasiveness:
Low. Feral and ruderal populations *G. hirsutum* or *G. barbadense* occur for example in Florida
(EPA, 2006) or Australia (Eastick, 2002; Eastick & Hearnden, 2006; OGTR, 2008). In Europe,
G. hirsutum has escaped from cultivation and was able to form feral populations in countries like
Greece or Italy. Also feral *G. herbaceum* populations can be found in southern Europe (see for
example Tutin et al., 1992).

Wild relatives with potential for invasiveness/weediness:
According to Manjunath (2005), *Gossypium tomentosum* is considered to be weed in agricultural fields in Hawaii.

Possible transgene-mediated fitness advantage:
It can be assumed that Bt toxin leads to fitness advantages for genetically engineered cotton. However, Eastick & Hearnden (2006) found no signs for increased weediness or invasiveness of Bt cotton.

Other information:
In Hawaii, commercial cultivation of genetically engineered cotton is prohibited because of possible hybridisation with wild cotton species *Gossypium tomentosum* (Hawkins et al., 2005). Transgenic cotton is also banned in some parts of Florida (EPA, 2006).

4.3 Case study: Genetically engineered maize in Mexico

Country: Mexico

Plant: Maize *(Zea mays)*

Mode of escape: Hybridisation with indigenous maize varieties

Region: Scientifically confirmed findings in Oaxaca, Mexico City, Guanajuato, Veracruz, Yucatán

Trait of escaped transgenes: Herbicide tolerance, insect toxicity

Genes: cry1Ab/c, cry9c, cp4epsps, and others

Description:

Mexico is the centre of origin as well as a centre of diversity for maize (*Zea mays*). There are several theories about the actual "birth place" of maize. There is strong evidence to suggest that it was first cultivated in the south of Mexico, in the federal states of Puebla, Mexico and Oaxaca (Serratos-Hernández, 2009). Current diversity is estimated to comprise several hundred maize varieties and landraces. All of them originate from teosinte, a wild grass species (OECD, 2003).

Figure 7

The presence of transgenic maize in Mexico was first revealed to the public through a study of 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 caused great surprise because at this time there were not even any small-scale field trials with transgenic maize in Mexico. According to ISAAA (2012), the first field trials were conducted in 2009. Although the findings from the study were immediately attacked by industry and industry affiliated scientists, they were consistent with tests conducted by Mexican authorities (Ezcurra et al., 2001). A working group of scientists commissioned by the North American Free Trade Agreement (NAFTA) confirmed that the

contamination of Mexican maize varieties is a reality (CEC, 2004). In 2005, a study found no traces of genetically engineered maize in traditional Mexican maize varieties (Ortíz-García et al., 2005). It took several studies published years after the first scientific findings of Quist & Chapela to finally confirm the spread of transgenic constructs in traditional Mexican maize varieties (Serratos-Hernández et al., 2007; Piñeyro-Nelson et al., 2009; Dyer et al., 2009; Mercer and Wainwright, 2008).

Contamination is documented in the following locations:

❯ Sierra Juárez (Oaxaca), samples taken in 2000 (Quist & Chapela, 2001)

❯ nature protection area near Mexico City, samples taken in 2003 (Serratos-Hernández et al., 2007)

❯ Sierra Juárez (Oaxaca), samples taken in 2001, 2004 (Piñeyro-Nelson et al., 2009)

❯ Guanajuato, Veracruz, Oaxaca and Yucatán, samples taken in 2002 (Dyer et al., 2009)

Other scientific reports from governmental sources in Mexico and NGOs that were only partly published (see Mercer and Wainwright, 2008): according to these reports, introgression of transgenic maize was also found in other federal states such as Chihuahua, Morelos, Durango, Oaxaca, und Tlaxcala.

The 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 nor labelled as being genetically engineered. According to this theory, 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). Further, studies have confirmed that maize can hybridise with its wild ancestor teosinte (*Zea mays* ssp. *mexicana*) (Chavez et al., 2012; Ellstrand et al., 2007). According to Guadagnuolo et al. (2006), maize x teosinte hybrids might even have fitness advantages since they produce more seeds than wild teosinte. There can also be gene introgression from teosinte to maize.

A study trying to reconstruct possible ways of transgenes spreading into traditional maize seed systems found that there is still a great deal of uncertainty regarding the way transgenes have spread throughout Mexico. At least in some regions, the presence of transgenes in indigenous maize landraces is not compatible with applied models.
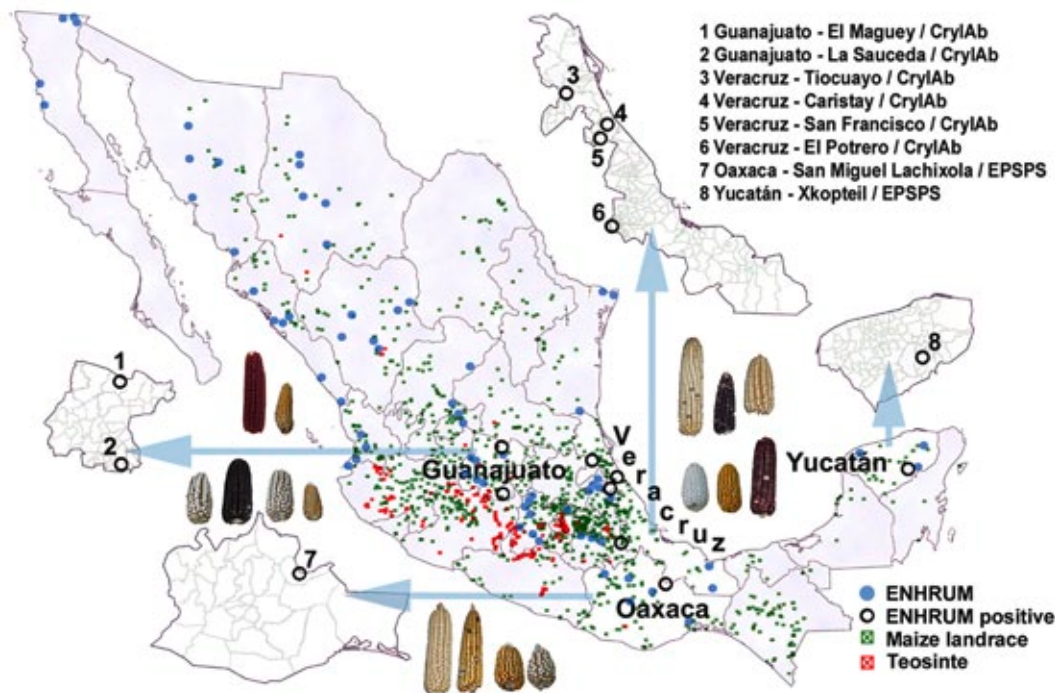
Figure 8 (Dyer et al., 2009, with kind permission): Distribution of survey sample and maize populations. ENHRUM (2002 Mexico Rural Household Survey) localities (blue), including those where transgenic proteins were detected (black circles). Distribution of teosinte (red) and maize landrace (green) according to INIFAP and CIMMYT genebank collections.

Biological profile: Maize *(Zea mays,* ssp. *mays)*

Family: True grasses (*Poaceae*)

Centre of origin/diversity:

Mexico is a centre of origin and diversity for maize (*Zea mays*). There are strong indications that maize crops were first cultivated in the south of Mexico, in the federal states of Puebla, Mexico and Oaxaca (Serratos-Hernández, 2009). Current diversity is estimated to comprise several hundred maize varieties and landraces. All of them originate from teosinte, a wild grass species (OECD, 2003).

Cultivated species: *Zea mays,* ssp. *mays*

Major producing countries:

In order of production volume: USA, China, Brazil, Mexico (FAOSTAT, 2013)

Spread of pollen:

Cross pollination, mainly by wind (OECD, 2003)

Spread of seeds:

Transport (OGTR, 2008)

Farthest pollen-mediated outcrossing distance measured to date:

4.440 meters (Bannert, 2006)

Seed persistence/dormancy:

Seeds that are lost during harvest may produce volunteers in following crop. However, seed dormancy is almost non-existent. Feral populations may appear under suitable climatic conditions (OGTR, 2008).

Potential hybridisation with other crop plants:

No known cases.

The so-called heterosis effect is often used in hybrid maize breeding. However, according to the biologist Allison Snow, such performance enhancing hybridisation effects still cannot be detected in crosses between traditional indigenous and genetically engineered maize. (in Gilbert, 2013).

Potential hybridisation with wild relatives:

Compatibility with most teosinte species, especially Mexican species *Zea mays* ssp. *mexicana* and *Zea mays* ssp. *parviglumis* (Wilkes, 1977; Baltazar et al., 2005; Chavez et al., 2012; Ellstrand et al., 2007).

Hybrids with *Tripsacum* species (*T. dactyloides, T. floridanum, T. lanceolatum, and T. Pilosum*) can be produced under artificial conditions. According to an industry dossier, there are related species in India that under controlled conditions can hybridise with maize (see Then, 2013).

Weediness / invasiveness:

Weediness not pronounced. However, maize is listed as a weed in the US (http://plants.usda.gov/java/invasiveOne?startChar=Z).

Wild relatives with potential for invasiveness/weediness:

Various teosinte species have weediness traits. In Mexico, for instance, Balsas teosinte (*Zea mays* ssp. *parviglumis*) and Mexican teosinte (*Zea mays* ssp. *mexicana*) are compatible with maize.

Possible transgene-mediated fitness advantage:

Almost no scientific data available. In general, the presence of Bt toxins in genetically engineered plants is regarded as a fitness advantage under pest infestation.

Further Information:

According to Guadagnuolo et al. (2006), maize x teosinte hybrids produce more seeds than wild teosinte and therefore have a fitness advantage. Teosinte species, on the other hand, can outcross into maize.

4.4 Case study: Genetically engineered oilseed rape in Canada

Country: Canada

Plant: Oilseed rape *(Brassica napus)*

Mode of escape: feral populations, hybridisation with *B. rapa*

Region: Provinces Manitoba, Saskatchewan, Québec; Vancouver

**Trait:** Herbicide tolerance (glyphosate, glufosinate)

Gene: cp4epsps, pat

Description:

In 1995, Canada was the first country to approve the commercial cultivation of genetically engineered herbicide tolerant oilseed rape (in North America, oilseed rape is called canola). Currently herbicide tolerant oilseed rape is grown on eight million hectares in Canada (ISAAA, 2012). Main cultivation areas are the provinces of Manitoba, Alberta and Saskatchewan. The spread of genetically engineered oilseed rape became public when a study claimed that nearly all of the conventional Canadian seed supply of oilseed rape contained transgenes (Friesen et al., 2003).

Figure 9

Several other studies published later on found that throughout the main cultivation areas feral populations had developed at the edges of fields and along roadsides. A major part of Canadian oilseed rape is exported (mainly to Japan) so seeds have to be transported over long distances to overseas ports such as Vancouver). Accordingly, populations of herbicide tolerant oilseed rape were found in the area around Vancouver (Yoshimura et al., 2006). Knispel et al. (2008) showed that 88 percent of feral oilseed rape populations examined in the province of Manitoba contained glyphosate tolerant plants. 81 percent were glufosinate tolerant. About 50 percent of the plants were tolerant to both herbicides. Tests revealed that hybridisation had almost no influence on fitness (Simard et al., 2005). According to the authors, populations are able to sustain themselves due to large-scale cultivation that leads to gene flow from transgenic oilseed rape. In a follow up to their study, Knispel & McLachlan (2010) found that between 93 and 100 percent of feral oilseed rape plants along field edges or roadsides in Manitoba tested positive for transgenic constructs. According to another publication, feral genetically engineered oilseed rape is also present in Québec. Additionally, all feral populations that were tested contained hybrids with *Brassica* rapa (Simard et al., 2006). Persistence of such hybrid populations over time was affirmed by Warwick et al. (2008). This long-term survey showed that feral hybrid populations of *B. napus* x *B. rapa* had decreased fitness, but nonetheless persisted over six consecutive years. Spread of transgenes by way of *B. rapa* is probable in eastern Canada because of the extensive cultivation of commercial varieties of this *Brassica* species (Warwick et al., 2003).
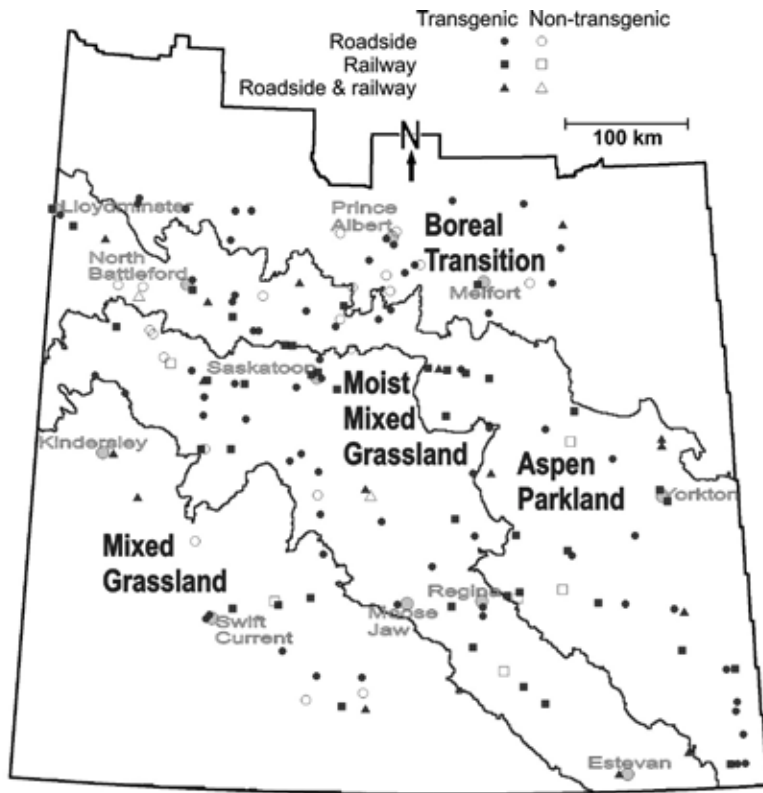
Figure 10 (Yoshimura et al., 2006, with kind permission): Occurrence of transgenic oilseed rape (glyphosate- or glufosinate-resistant) in ruderal areas (along railways or major roads) across Saskatchewan ecoregions (semi-arid Mixed Grassland to subhumid Aspen Parkland and Boreal Transition) in 2005.

4.5 Case study: Genetically engineered rape in the US

Country: USA

Plant: Oilseed rape *(Brassica napus)*

Mode of escape: Ruderal populations

Region: North Dakota; California

Traits: Herbicide tolerance (glyphosate, glufosinate)

Gene: cp4epsps, pat

Description:

Figure 11

Oilseed rape (canola) is only cultivated in a few federal states in the US. The total cultivation area is 1.6 million hectares. 1.3 million hectares are in the state of North Dakota (NASS, 2012). Small areas are cultivated in Idaho, Minnesota, Montana, Oklahoma, Oregon and Washington. Commercial cultivation of genetically engineered oilseed rape started in 1998 and, at present, accounts for more than 90 percent of all oilseed rape fields in the US. The very first study in the USA on unintended dispersal demonstrated large-scale dispersal of herbicide tolerant oilseed rape along roadsides in North Dakota (Schafer et al., 2011). 80 percent of all oilseed rape plants

growing along roadsides tested positive for genetic modification. Half of the plants contained the cp4epsps gene for glyphosate tolerance. The other half contained

 the pat gene that makes the plants tolerant to glufosinate. Some of the plants were tolerant to both herbicides. Ruderal populations were found mostly near storage facilities or grain elevators, but also along railway tracks. There have, as yet, been no follow up studies on persistence under regional conditions or introgression into wild relatives such as *B. rapa*.

Ruderal populations of glyphosate tolerant oilseed rape were also detected in California where variety trials with transgenic oilseed rape had been conducted (Munier et al., 2012). Transgenic plants were found along roadsides and in the vicinity of places where the combine harvester had been emptied.
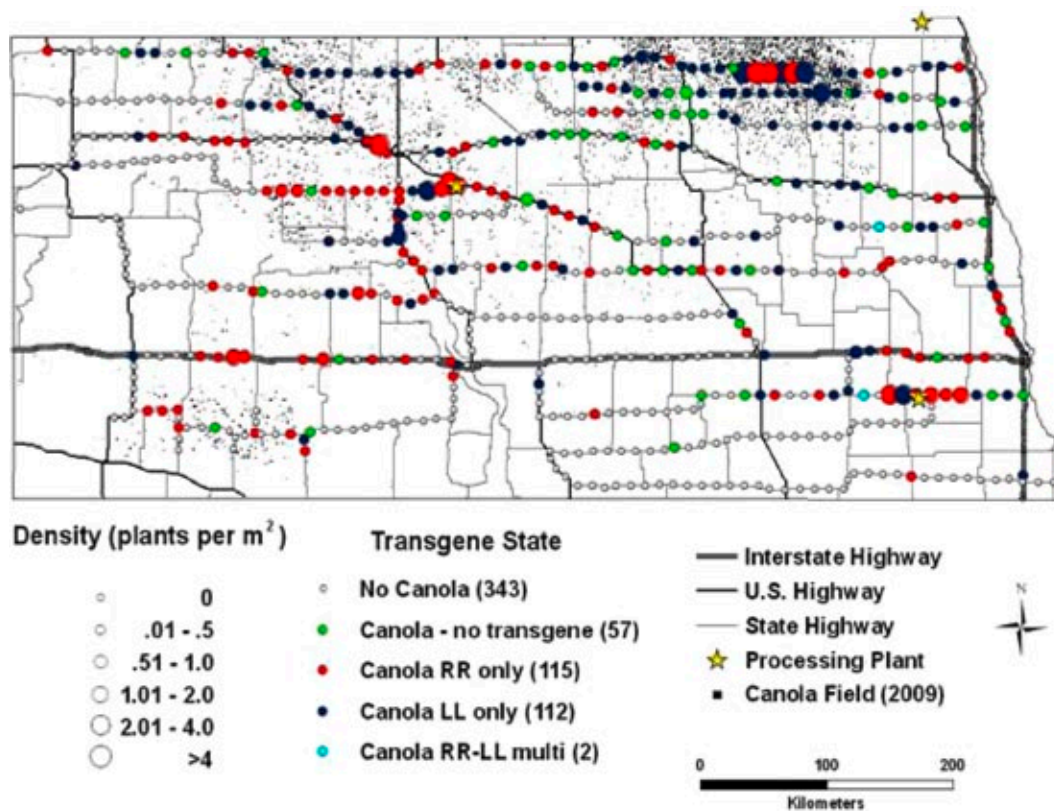
Figure 12 (Schafer et al., 2011, with kind permission): Distribution and density of feral canola populations in North Dakota road surveys (2010). Transgenic plants are resistant to glyphosate (RR) or glufosinate (LL) or to both herbicides.

4.6 Case study: Genetically engineered oilseed rape in Japan

Country: Japan

Plant: Oilseed rape *(Brassica napus)*

Mode of escape: Ruderal populations, hybridisation with *Brassica rapa*

Region: Proximity of ports and along transportation routes

Traits: Herbicide tolerance

Gene: cp4epsps, pat

Description:

Oilseed rape (*B. napus*) was introduced in Japan in the 19th century where even today its cultivation is of only minor importance. However, there is large-scale cultivation of related *Brassica* species such as *B. rapa* and *B. juncea*, which easily hybridise with oilseed rape. The cultivated forms of the two species are used as root or leafy vegetables. Both species are present as wild species or feral forms of cultivated varieties of *B. rapa* and *B. juncea*. For example, monitoring of port areas showed that feral oilseed rape coexists with wild populations of *B. juncea* (Kawata et al., 2009). Japan is

Figure 13

one of the main importing countries for genetically engineered oilseed rape. About 90 percent of two million tons that are imported per year stem from Canada. In Canada, more than 90 percent of cultivated oilseed rape is genetically engineered to tolerate herbicides like glyphosate. 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. Aono et al. (2006) detected transgenic oilseed rape plants that had hybridised with each other and were tolerant to both herbicides.

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). 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. The first transgenic hybrid plants between *B. napus* and *B. rapa* was found in Yokkaichi (Aono et al., 2011).

According to research, the properties of feral transgenic oilseed rape plants might have changed under the influence of climatic conditions. From an ecological perspective, it should be of concern that some larger than normal plants were found. These plants have also become perennial (Kawata et al., 2009) whereas oilseed rape and all other *Brassica* species growing in Japan are annual.

4.7 Case study: Genetically engineered oilseed rape in Australia

Genetically engineered oilseed rape has been cultivated in Western Australia since 2009. Western Australia is a so-called GMO free region, and the cultivation of transgenic crops is only possible as an exemption. Accordingly, herbicide tolerant oilseed rape is cultivated only in distinct areas (less than ten percent of overall oilseed rape acreage, according to McCauley et al., 2012).

Nonetheless, there are first, non peer-reviewed reports of ruderal transgenic oilseed rape along roadsides in Western Australia. Tests undertaken by a nature conservation organisation revealed that more than 60 percent of samples taken in October 2012 contained transgenic constructs making the plants tolerant to glyphosate (CCWA, 2012). According to the organisation, one of the main problems regarding genetically engineered crops in Australia is political negligence and an unwillingness to engage with the issue of their side effects. There is therefore no official monitoring programme or guidelines on how to manage ruderal transgenic populations.

Figure 14

4.8 Case Study: European Union

In Germany (as well as in many other European countries), field trials with genetically engineered oilseed rape were conducted from the 1990s onwards. Many trials were multi-year and large scale. For many years they were conducted under conditions that made escape from the fields very probable:

> there was no transparency with regard to the trial sites;

> there was no monitoring beyond the fields;

> the isolation distances to other oilseed rape fields were too short (100 or 200 meters in trials with no buffer strips) (Arndt & Pohl, 2005);

> requirements for isolation distances or buffer strips imposed by the competent authority sometimes changed from year to year (Arndt & Pohl, 2005);

> companies did not properly implement requirements (Arndt & Pohl, 2005);

> there was no information for bee keepers.

As yet, no systematic attempt has been made to determine the consequences of these field trials regarding possible persistence of transgenic oilseed rape in the environment. Control samples from several ruderal populations of oilseed rape in areas close to selected field trial sites in southern Germany did not show positive results (Franzaring et al., 2007). However, feral genetically engineered oilseed rape has been found in North Rhine-Westphalia at a distance of 700 meters from a former trial field (Hofmann & Neuber, 2007).

In Sweden, transgenic oilseed rape was even found ten years after experimental field trials (D'Hertefeldt et al., 2008).

According to the EU Commission, contaminations with Bayer transgenic oilseed rape are found regularly (see below). In 2007, genetically engineered oilseed rape Ms1xRf1, Ms1xRf2 and Topas lost market authorisation. These plants were authorised for seed production but never grown on a large scale in the EU. Nevertheless, the EU Commission had to establish a specific regulation for the removal of the plants from the environment. Contaminations were allowed with the oilseed rape provided it did not exceed 0,9% for a period of five years. This period had to be prolonged in 2012 for another five years because minor contaminations were still being reported. This example shows that even in cases

Figure 15

with low-level contaminations a long period of time is needed to minimise the uncontrolled dispersal of genetically engineered oilseed rape in relevant products. It is doubtful whether transgenic oilseed rape can be removed from the environment at all after being grown on large scale as it is in Canada and the US.
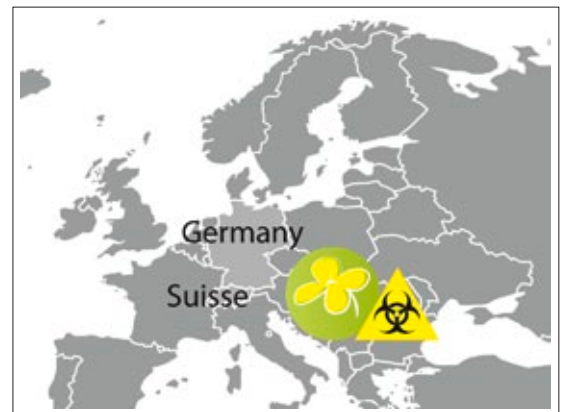
Documetation: Specific regulations for the removal of transgenic oilseed rape owned by company Bayer as put in place by the EU Commission.

Commission Decisions 2007/305/EC (2), 2007/306/EC (3) and 2007/307/EC (4) set out the rules for the withdrawal from the market of the GM material: Ms1xRf1 (ACS-BNØØ4- 7xACS-BNØØ1-4) hybrid oilseed rape, Ms1xRf2 (ACS- BNØØ4-7xACS-BNØØ2-5) hybrid oilseed rape and Topas 19/2 (ACS-BNØØ7-1) oilseed rape, as well as their derived products.

All three Decisions provided for a transitional period of time of years, during which food and feed containing the GM material were allowed to be placed on the market, in accordance with Article 4(2) or Article 16(2) of Regulation (EC) 1829/2003, subject to a number of conditions. The Decisions require in particular that the presence of the GM material in food and feed does not exceed a threshold of 0,9 % and that the presence of this GM material be adventitious or techni-cally unavoidable.

Recent test results notified by stakeholders to the Commission show that at the end of this 5 year period the measures undertaken by the authorisation holder have allowed the removal of nearly all the GM material from the market. However, these results also show that minute traces (< 0,1 %) of the GM material may still be present in the food or feed chain at the end of the transitional period set out in Decisions 2007/305/EC, 2007/306/EC and 2007/307/

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The presence of remaining traces after the expiry date set out in these decisions, despite the measures undertaken by the notifier, can be explained by the biology of oilseed rape which can remain dormant for long periods as well as by the farm practices which have been employed to harvest the seed and resulting accidental spillage, the level of which was difficult to estimate at the date of adoption of the three above mentioned Decisions.

It was therefore considered necessary to extend the transitional period of time for another 5 years, that is until 31 December 2016. This supplementary transitional period should provide sufficient time to allow the total removal of the GM material from the food and feed chain.

Source: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

4.9 Case study: Genetically engineered rape in Switzerland

One of the few empirical studies on transportation losses of genetically engineered oilseed rape in Europe was recently conducted in Switzerland (Schoenenberger & D'Andrea, 2012). 2400 samples were taken along railway tracks throughout Switzerland. 50 samples proved positive for the presence of an enzyme that is characteristic for Roundup Ready plants and makes them tolerant to herbicides with the active ingredient glyphosate. The high number of genetically engineered oilseed rape plants found in the samples in 2011 and 2012 was remarkable because the import of transgenic oilseed rape has been prohibited in Switzerland since 2008. Only small traces of contamination of less than 0.5 percent are allowed. 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 offered them selective advantages. Another study (Hecht et al., 2013) confirmed these findings and identified loading terminals and stations as key risk areas where there was a higher concentration of transgenic plants.

Figure 16 (Schoenenberge & D'Andrea (2012), with kind permission): Investigated railway stations and yards in Switzerland. Grey dots: negatively tested sites; Black dots: positively tested sites (Lugano and Basel); X: railway areas where no oilseed rape was detected.

Biological profile: Oilseed rape *(brassica napus)*

Biological profile: Oilseed rape

Family: *Brassicaceae*

Centre of origin:

Oilseed rape originates from a natural crossing of *B. oleracea* and *B. rapa*, which probably happened in the Mediterranean or Atlantic areas of Europe less than 10.000 years ago

Related cultivated species:

B. oleracea (cabbage, chinese kale, broccoli, cauliflower, ...), *B. rapa* (pak-choi, turnip rape, …) (OECD, 2012)

Major producing countries: EU, China, Canada, India (FAOSTAT, 2013)

Spread of pollen:

Mainly insect pollination, but also by wind (OECD, 2012)

Farthest pollen-mediated outcrossing distance measured to date:

26 kilometres, male sterile plants were used in this field trial (Ramsay et al., 2003)

Fertility of pollen: 4-5 days under natural conditions (OECD, 2012)

Seed persistence/dormancy:

Empirically proven to date: Over 11 years Lutman et al. (2003). Oilseed rape has a high potential for volunteer plants.

Potential for hybridisation with other crop plants:

Oilseed rape can hybridise with cultivated *B. rapa* and *B. oleracea* varieties; hybridisation with *B. rapa* is more probable (Devos et al., 2009). The relatives of *B. napus* can be illustrated as the so-called "triangle of U" (see figure).

Hybridisation with wild relatives:

In Europe, oilseed rape can hybridise with the following wild or feral relatives (OECD, 2012; Devos et al., 2009):

> *Brassica rapa*

> *Brassica juncea*

> *Brassica oleracea*

> *Brassica nigra*

> *Diplotaxis muralis*

> *Diplotaxis tenuifolia*

> *Erucastrum gallicum*

> *Hirschfeldia incana*   > *Sinapis alba*

> *Raphanus raphanistrum*   > *Sinapis arvensis*
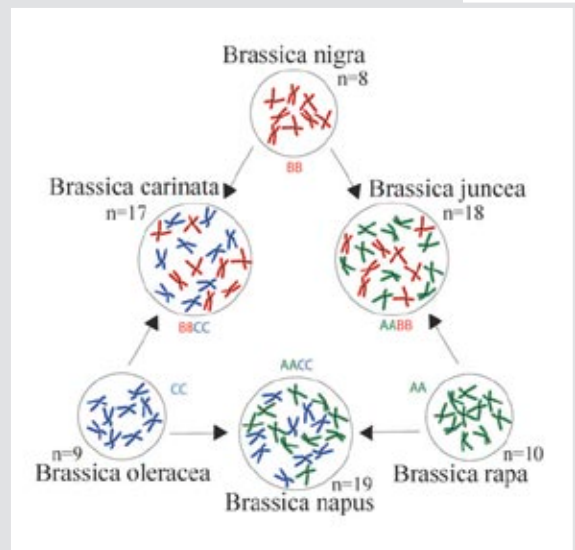
http://en.wikipedia.org/wiki/Triangle_of_U

Weediness / invasiveness:

All cultivated *Brassica* species are also weeds. Oilseed rape can appear in ruderal populations along field edges and roadsides. Pivard et al. (2008) found that ruderal populations are self-sustaining in a semi permanent form. According to Munier et al. (2012), herbicide tolerant oilseed rape is weed.

Wild relatives with potential for invasiveness/weediness:

There are weedy forms of *B. rapa* and B. olereracea. Weedy *B. rapa* is found worldwide (OECD, 2012). Also the wild relative species *Sinapis arvensis*, *Raphanus raphanistrum* and *Hirschfeldia incana* are considered to be weeds.

Possible transgene-mediated fitness advantage:

Hybrids between *B. napus* and *B. juncea* (Di et al., 2009) as well as *B. napus* and *B. rapa* (Rose et al., 2009) showed only a slight loss in fitness when compared with the wild species. Tests with Bt oilseed rape showed that the plants have fitness advantages when under pest insect pressure. (Mason et al., 2003). When sprayed with herbicide, relevant herbicide tolerance will lead to increased possibilities of spreading. According to Claessen et al. (2005) transgenic modifications for modified oil content (such as a higher content of stereat or laurat) provide oilseed rape with fitness advantages. Simulations show that related wild species such as *B. rapa* und *Raphanus sativus* most probably have higher fitness with the introgression of Bt genes through hybridisation (Letourneau & Hacker, 2012). This might also be the case for *Raphanus raphanistrum* (Meier et al. (2013).

4.10 Case study: Bt poplar in China

Country: China

Plant: Black poplar (*Populus nigra*), hybrids of white poplar (*P. alba* × [*P. davidiana* + *P. simonii*] × *P. tomentosa*)

Mode of escape: Outcrossing

Region: Northern and Eastern China

Trait: Insect toxicity (Bt)

Genes: CryIAc and others

Description:

According to the FAO (2009), China is home to the largest poplar (and willow) plantation area worldwide. About 85 percent of forest acreage of these two species is cultivated in China. Poplar plantations cover an area of six million hectares (Lu et al. 2006).

Genetically engineered black poplars (*P. nigra*) have been cultivated commercially in China since 2001. The trees produce the insecticidal protein CryIAc. In 2011, the official acreage of Bt poplar was 495 hectares (Lu & Hu, 2011). Between 2001 and 2003 a further 400.000 white poplar hybrids (called hybrid poplar 741) that also produce Bt toxins (Cry1 and API) were planted. According to available information, these trees are sterile (Wang, 2004).

Figure 17

In 2004, the first published reports appeared on transgene introgression into neighbouring poplar plantations in the North-Western province of Xinjiang, where field trials with Bt poplars started in 1994 (Pearce, 2004). A first systematic study tried to determine outcrossing into two neighbouring poplar plantations (Lu et al, 2006). Hybrids of Bt black poplar and *Populus pioner* were found at a distance of 500 meters. However, DiFazio et al. (2012) found much larger outcrossing distances in poplar in Canada. The authors also assume that Bt genes lead to fitness advantages in transgenic poplar and increase the dispersal of transgenic varieties.

The situation in China is problematic in that there have been no published scientific studies on the outcrossing of Bt poplar. Furthermore, there doesn't seem to be any kind of governmental oversight regarding usage or sale of genetically engineered poplar (such as cuttings). Gene flow from existing Bt polar plantations is also not monitored (see for example Pearce, 2004; Wang, 2004, Then & Hamberger, 2010). Possible problems of outcrossing were also mentioned by Ewald et al. (2006).

All poplar species are considered to be trees with a high potential for dispersal, as they are wind pollinated, produce large amounts of pollen, hybridise easily with each other, and proliferate vegetatively by cuttings and root suckers. Furthermore, China is known to be a centre of origin for several poplar species (FAO, 2009; Du et al., 2012).

Biological profile: Black poplar *(Populus nigra)*

Family: *Populus*

Centre of origin/diversity:

The *Populus* family comprises about 35 related species, most of them growing in the Northern Hemisphere. Centres of diversity are China and Central Europe, amongst others. Domestic species to Europe are black poplar (*Populus nigra*), silver poplar (*P. alba*), aspen *(P. tremula)* as well as natural hybrid grey poplar (*P. canescens= P. alba x P. tremula*) (Aas, 2006).

Cultivated species:

In Europe, domestic poplar species as well as balsam poplar (*P. balsamifera or P. trichocarp*a) and hybrids of carolina poplar (*P. x canadensis*) (originally a hybrid of European black poplar and *Populus deltoides* from Northern America*)* are used commercially (plantations) (Aas, 2006).

Major producing countries:

see above

Spread of pollen:

Wind (see for example Testbiotech, 2010, Schütt et al., 1994; diFazio et al., 2012)

Spread of seeds and other ways of reproduction:

Poplar species produce large amounts of light and cotton-like seed wool. Seeds are dispersed by water and wind. Poplar also reproduce by root suckers as well as branches and cuttings (Schütt et al., 1994; Testbiotech, 2010).

Farthest pollen-mediated outcrossing distance measured to date:

Pollen or seed mediated outcrossing in *P. nigra*: > 2 kilometres (Bialozyt, 2012; Rathmacher et al., 2010). Outcrossing in *P. trichocarpa* 7.6 kilometres (Slavov et al., 2008).

Seed persistence/dormancy:

No dormancy in *P. nigra* (Lefèvre et al., 2001).

Hybridisation potential with other crop plants:

Poplar species hybridise with each other frequently (Aas, 2006). In the course of evolution, some hybrids have become separate species. Many hybrids of *P. nigra* and species like *P. deltoides,. trichocarpa or P. x canadensis* are cultivated or are growing in natural habitats. This process is described as "introgressive hybridisation" which means that other poplar species mix with black poplar and there is a constantly connected "hybrid swarm" which leads to a condition where clear boundaries between the original species are no longer possible (Aas, 2006). This is one of the reasons why the original black poplar is listed as an endangered species.

Possible transgene-mediated fitness advantage:

Under controlled conditions, Bt poplar hybrids *Populus tremula* × *Populus tremuloides*) had fitness advantages under pest insect pressure compared with the wild type (Hjältén et al., 2012).

Other information:

Compared with cultivated crops, all trees have high potential for dispersal over a long time:

> trees are main constituents of ecosystems,

> forests are the oldest existing ecosystems,

> many trees live for a very long time (hundreds or thousands of years) and stay fertile during their lifetime (*P. nigra* can live up to 300 years)

> most tree species disperse pollen and seed over long distances, often in large quantities and cycles,

> willows and poplars that grow on the lowlands of large flood plains or river valleys can reproduce for example via root suckers or bits of branches that are then transported for long distances along the river.

> root suckers live for a very long time: the root colony (clone colony) of a specific individual (Pando) of aspen (*Populus tremuloides*) whose trunks are interconnected via roots and cover an area of 43 hectares is estimated to be 80.000 years old (http://en.wikipedia.org/wiki/Pando_%28tree%29),

> tree pollen can easily reach high layers of air and thus be dispersed over longer distances than pollen from cultivated crops.

4.11 Case study: Bt rice in China

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 trace amounts of non-approved transgenic rice in 16 of 561 samples of imported foodstuff from China (BVL, 2012).

Figure 18
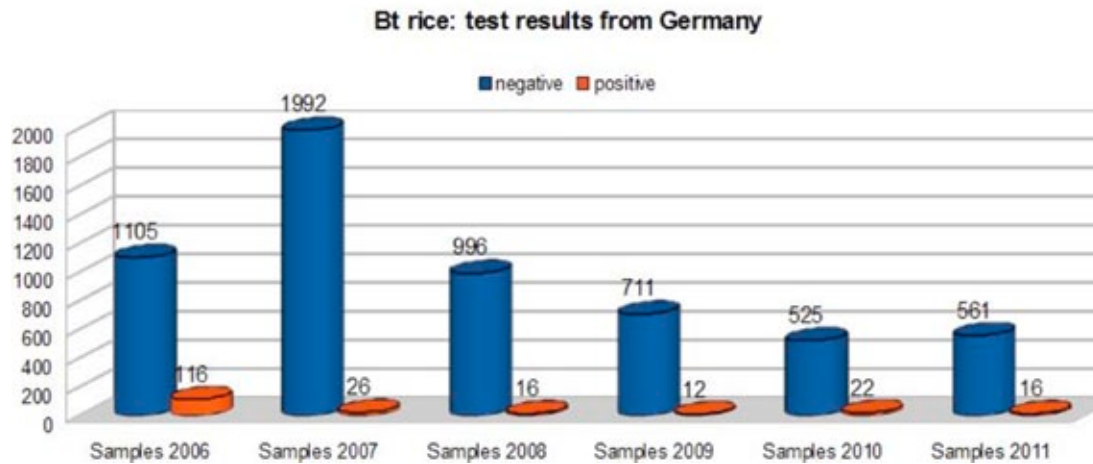
Figure 19: Results from test of rice imports in Germany and findings of non authorised genetically engieered rice form China, Source: BVL, 2012

According to the EU Rapid Alert System for Food and Feed (RASFF, 2013) in 2013, most cases of unapproved GMOs into the EU are still due to transgenic rice from China.

Even though there are still no studies on the background and dimension of the spread of Bt rice through the rural seed systems in China, there are indications that transgenic rice has permanently introgressed into Chinese rice seed systems. Hybridisation with weedy rice may be one of the ways in which transgenic rice is spreading.

If genetically engineered rice is planted in regions where wild rice grows as weedy rice, transgenes can cross-pollinate with wild or weedy forms. Wild rice is widespread in many rice cultivation areas (Ferrero, 2003; Chen et al., 2004) and gene flow between wild and cultivated rice forms growing in vicinity is extensive (Chen et al., 2004).

According to RASFF (2013), Bt63 is even found in rice products from Pakistan, indicating that transgenic rice has escaped over country borders.

Biological profile: Rice *(Oryza sativa)*

Family: True grasses (*Poaceae*)

Centre of origin/diversity:

According to new research, the Pearl River region in South China is the centre of origin for cultivated *Oryza sativa* rice. (Molina et al., 2012). There is a further important cultivated rice species called *Oryza glaberrima* which originates from Niger delta.

Cultivated species:

> *Oryza sativa* (there are several so called ecotypes, grains of *japonica* varieties are shorter, broader, softer and more sticky than *indica* varieties)

> *Oryza glaberrima* (African rice)

> hybrid of *Oryza sativa* X *Oryza glaberrima (OGTR, 2005)*

Major producing countries:

China, India, Indonesia, Bangladesh, Vietnam, Thailand, Pakistan, Philippines, Japan. Around 90 percent of global rice production comes from Asia. (OGTR, 2005).

Spread of pollen:

Cultivated rice is mostly autogamous (self-pollinated), but pollen is also spread by wind and bees (OGTR, 2005). Wild rice is mostly cross-pollinated and wild rice pollen is viable for twice as long as pollen from cultivated species.

Spread of seeds:

Cultivated varieties of rice hardly spread at all through seeds because the grains of most varieties stick to the spindle until harvest and are therefore immobile. Rice plants can propagate vegetatively through new growth after harvesting, so that under favourable conditions, there may even be a second harvest (see OGTR, 2005). Oryza species from South America even disperse via rivers. Broken shoots can be carried along by the river water and grow roots when they reach land again.

Farthest pollen-mediated outcrossing distance measured to date:

110 meters (Song et al., 2004); pollen flow was detected at a distance of 250 meters (Kenya et al., 2009).

Seed persistence/dormancy:

Cultivated rice stays fertile for two or three years in the fields. Weedy rice was found to germinate after ten years in soil (Ferrero, 2003).

Hybridisation potential with other crop plants and weedy relatives:

Gene flow between wild and cultivated rice forms growing in vicinity is extensive (Chen et al., 2004, Lu & Yang 2009).

Sexual compatibility between wild and cultivated rice is used in breeding to introduce genes that confer resistance in wild rice into cultivated varieties. In the past, this method has been used for *O. nivara* and *O. longistaminata.* Other compatible wild species are *O. meridionalis* and *O. rufipogon.*

Weediness / invasiveness:

A biological characteristic of rice is the hybridisation dynamic between cultivated rice, feral rice and wild species. Hybrids often emerge as weedy rice. Weedy rice has fitness advantages compared to cultivated rice and often has increased tolerance towards drought, cold or wet conditions (Vaughan & Morishima, 2003). In the history of rice as a food plant, there have been several cases where rice has turned wild and then been re-domesticated (Vigueira et al., 2013)

Wild relatives with potential for invasiveness/weediness: see above

Possible transgene-mediated fitness advantage:

Research has shown that Bt rice (Fu Li et al. 2012) as well as HT rice (Wang et al., 2013) can achieve fitness advantages. Field trials have shown that weedy rice has increased fitness after the introgression of Bt genes from cultivated rice (Lu & Yang 2009). There was also an increase of Bt content in some hybrids of wild rice and cultivated rice (Xia et al., 2009).

4.12 Other cases

There are already several cases where the release of genetically engineered organisms could have led to the uncontrolled spread of transgenes into the environment, without there being any in-depth investigations.

›	**Genetically engineered papaya (Thailand, Hawaii)**
	The cultivation of transgenic papaya is prohibited in Thailand. Nonetheless, papaya with transgenic constructs for resistance to *papaya ringspot virus* (PRSV) is regularly found in fruits imported from Thailand (Davidson, 2008; RASFF, 2013).
	The source of the contamination is probably a state research station, which apparently gave transgenic papaya seeds to farmers at the beginning of the year 2000. Pollination could have taken place ever since. Tests carried out in 2005 revealed transgenes in a third of all papaya plantations in the eastern province of Rayong and the northeastern provinces of Mahasarakham und Chaiyaphum Kalasin (Greenpeace, 2012). The presence of genetically engineered papaya in Thailand was confirmed about ten years ago but efforts to control transgene dispersal have obviously not been successful. Papaya can also grow wild (OECD, 2005; OGTR, 2003).
	Hawaii has also reported the uncontrolled spread of transgenic papaya. (Bondera & Query, 2006). In 2006, tests performed by the NGO *Hawaii Seed* revealed that about half of the samples contained transgenic contaminations.

›	**Genetically engineered maize, oilseed rape, cotton and soybean in South Korea:**
	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 (see graphic below).

According to the report, besides a few isolated plants they found whole populations of transgenic plants close to some of the farms. Previous studies showed occurence of genetically engieered plants around import harbours (Lee et al., 2009; Park et al., 2010). Investigation in molecular biology found epigenetic changes in the plants (Waminal et al., 2013).

Future cases

Some examples of genetically engineered products under discussion that might be the cause of uncontrolled environmental dispersal of transgenes in near future:

> Golden Rice in the Philippines, Bangladesh and India
> Bt-aubergine in India
> genetically engineered trees in the US and the EU
> genetically engineered sorghum in Africa
> genetically engineered maize in Africa
> genetically engineered alfalfa in the US
> genetically engineered salmon in Canada
> genetically engineered olive flies in Spain.

5. Discussion

The case studies show that globally there are already many cases of genetically engineered crops moving spontaneously beyond the field sites into the environment. Some of these plants are likely to have escaped any kind of control and are able to persist permanently and even proliferate. In several cases, there is very little knowledge of the actual situation and many questions remain. There has also been no detailed investigation into the routes of dispersal or the true potential for proliferation and interactions with the environment. The following table gives an overview of current knowledge.

Plant species	Country	Commercial cultivation	Cases in field sites	Cases beyond field sites	State of research	Likelihood of persistence and invasiveness in the regions concerned
Creeping bentgrass *Agrostis stolonifera*	US	No	n.i.	Yes	Confirmed by several publications	Very likely to persist and invade because bentgrass shows invasive potential.
Black poplar *Populus nigra*	China	Yes	Yes	n.i.	Conclusive studies still missing	Only very few investigations about potential gene flow into wild poplar.
Cotton *Gossypium hirsutum*	Mexico	Yes	n.i.	Yes	Pilot study	Very likely to persist because of gene flow into wild relatives.
Maize (corn) *Zea maize*	Mexico	No	Yes	n.i.	Confirmed by several publications	Persistence very likely because of informal seed exchange and the gene flow between fields.
Oilseed rape *Brassica napus*	Australia	Yes	Yes	Yes	Conclusive studies still missing	No conclusive publications.
Oilseed rape *Brassica napus*	Canada	Yes	Yes	Yes	Confirmed by several publications	Persistence and further spread very likely because of confirmed gene flow to related species.
Oilseed rape *Brassica napus*	Europe	No	n.i.	Yes	Relevant studies are mostly missing	No information about crosses into wild relatives yet.
Oilseed rape *Brassica napus*	Japan	No	No	Yes	Confirmed in several publications	Gene flow into related species likely to have occurred.
Oilseed rape *Brassica napus*	US	Yes	Yes	Yes	Pilot study	No information about crosses into wild relatives. Unnoticed gene flow with wild species is likely.
Papaya *Carica papaya*	Thailand	No	Yes	n.i.	Conclusive studies still missing	Gene flow into de-domesticated papaya could cause persistence.
Rice *Oryza sativa*	China	No	Yes	n.i.	Conclusive studies still missing	Persistence is likely due to informal seed exchange and gene flow with weedy rice.

n.i= not investigated

Current gaps in research

Despite strong political support for the commercial cultivation of genetically engineered plants in some countries, there has been very little investigation into the proliferation of these plants in the environment. This is mirrored in the low overall number of publications.

> The first study on the spread of transgenic oilseed rape plants in the US was published in 2012, about 15 years after it was first grown there. (Schafer et al., 2012); Mexico is a centre of origin for most commercially grown cotton varieties. Cotton has been grown there for many years, but it was not until 2012 that a study on the presence of transgene constructs in wild cotton populations was carried out.(Wegier et al., 2012);

> There are only very few empirical studies on the problems associated with transgenic maize out-crossing into the traditional varieties of Mexican maize (see for example Quist & Chapela, 2001; Piñeyro-Nelson et al., 2009; Dyer et al., 2009). Relevant scientific questions such as the enhanced fitness of Bt maize under regional conditions have not been addressed at all.;

> China is one of the centres of genetic diversity for black poplar. Although genetically engineered Bt poplar have been grown here for the past ten years there has been little or no research into exactly where they are grown or their spread into wild poplar. (see Then & Hamberger, 2010a);

These gaps in scientific research highlight a lack of knowledge of the impact of commercial cultivation but also proliferation due to experimental field trials or the import of viable seeds for food and feed production. For example, EU import statistics since 2005 have shown that non-authorised bt rice is present in imports from China. There does not however seem to be any systematic research in this area.

There further does not appear to be very much research going on in the EU on relevant issues such as the spread of transgenic oilseed rape from the field trials or seed cultivation sites. Europe also needs systematic monitoring of imports. Switzerland is ostensibly free of genetically engineered plant technology, but findings of transgenic oilseed rape along its railway lines, highlight the need for monitoring imports in Europe (Schoenenberger & D'Andrea, 2012)

A comparison with risks of alien species

There many unresolved issues on the short-, medium- and long-term impact on the environment. Some consequences of the uncontrolled spread of transgenes are obvious:

The control of weedy species can become more complicated when genetically engineered plants with herbicide tolerance proliferate increasing both the pesticide load in the environment and the workload of farmers. Operational costs may also increase.

Genetically engineered organisms, which are no longer allowed on the market for economic or ecological reasons, cannot be removed efficiently if they propagate, crossbreed and spread spontaneously into the environment. They can also contaminate harvests and cause substantial economic damage.

The long-term impact on ecosystems is hard to predict. In this regard, transgenic plants can be compared to alien species. Invasive species can replace a single species or a whole range of species. Further, they can change the conditions within ecological systems. There some are well- documented crosses between alien species and the original species. Crossing can cause losses in the genetic information of the original species, a reduction in genetic diversity and an ongoing incremental change of genetic identity in the original plants[5]. It is hard to predict which species will become invasive:

> *"Only 10 percent of the species can persist in a new region, disappear and come back on other places (impermanent persistent species). But in case of favourable biotic and abiotic conditions, the alien species can become permanent persistent. Under these conditions, the rate of success is around 20 percent of the imparment persistent species in disturbed ecosystems and around 10 percent in more naturally conserved ecosystems, which means an overall rate of success around 2-1% of all alien species (...). Again 10 percent from these permanent persistent species can become invasive (which means less then 0,2% of all alien species). [translation] (BfN, 2005).*

Some characteristics of particular species can render higher fitness so they might e.g. produce a higher number of seeds, grow faster, or be resistant to biotic or abiotic stressors. Further plasticity of the genome, based on epigenetic mechanisms can enable quick adaption to new environmental conditions and therefore foster invasiveness of species (BfN 2008). Epigenetic mechanisms can change gene-regulation in the plants for example by silencing DNA sequences (see Moch, 2006) and therefore allow much quicker adaption of the plants to environmental conditions than would be expected through mutation and selection.

Even if the biological characteristics of a species are known, its potential to persist or invade in new environmental conditions very often cannot be predicted. Some of the alien species only persist in distinct local regions and do not spread substantially over a longer period of time (i.e. lag-phase) but even after many years, they may still become invasive. It is also difficult to predict the ecological impacts of invasiveness (BfN, 2005). The fact that climate change and disturbed ecological systems can foster invasiveness could cause even further uncertainty.

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5   www.floraweb.de/neoflora/auswirkung.html

Specific risks of genetically engineered plants

The comparison between the spread of genetically engineered organisms and the invasive potential of alien species also shows major differences. In the case of genetic engineering, one must consider both the adaption and spread of a new species within an ecosystem and the spread of technically inserted genetic information within the pool of genes of a species that has already adapted to its environment. The dynamics of proliferation within established species can be completely different from the pattern of the ecological potential of alien species within a new environment. Possible consequences are uncontrolled spread of (undesirable) new genetic potential within the gene pool of a species (such as production of insecticidal proteins) that can cause substantial ecological problems in the long term.

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 promotors and stop codons, that are not subject to the natural 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 imply new risks for the environment.

Relevant risks are proliferation of plants pests (if genetically engineered plants or its 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.

There are also significant differences when it comes to the issue of spatio-temporal control, human responsibility and regulatory issues. While invasive spread of alien species is the result of evolutionary processes and in most cases are not driven by deliberate decisions, genetically engineered plants are made by mankind and their characteristics are a novum within the ecosystems. Its proliferation into the environment is not caused by inevitable global processes, but in most cases and at least at some stages it is based on deliberate decision making. Responsibility for the release of these plants can only be implemented if future development can be predicted in a reliable way and possible adverse impacts can be avoided by effective controls.

The long-term ecological consequences of releases of genetically engineered plants that escape spatio-temporal control can hardly be predicted. In this case, risk assessment needs to take into account evolutionary dimensions. Evolutionary processes make it possible that events with a low probability have a reasonable chance of occurring. According to Breckling[6] the following impact factors have to be considered:

 "– Evolutionary dynamics combine large numbers on the population level and singularities on the molecular scale;

 – Even combinations with extremely low probability have a reasonable chance to occur;

 – Depending on the particular environmental conditions organismal reproduction enables selfamplification across several orders of magnitude and large-scale dispersal and cannot be predicted;

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6   GMLS Konferenz in Bremen, 2012, http://www.gmls.eu/

– *Genetic drift can cause the fixation of genes on pure random basis particularly in small populations;*

– *The fitness of new genomic constituents cannot be calculated in absolute terms. It depends on the environment and its future changes."*

The further development of environmental conditions and the long-term ecological behaviour of genetically engineered plants cannot be reliably predicted at the time they are released. In consequence, this means if spatio-temporal control is not possible, the necessary prerequisites for reliable risk assessment are not given.

The risk of outcrossing into wild species could be enhanced by climate change. There are cases published showing that especially hybrids of cultivated species with wild species develop a higher fitness under stress (Mercer et al., 2007). A higher gene flow for oilseed rape under extreme climatic conditions has also been reported (Franks & Weis, 2009). Their study shows there was a change in the time for flowering resulting in matching of flowering between species.

6. Precaution and spatio-temporal control

On the basis of the documented cases and current gaps in knowledge regarding dispersal, interactions with the environment and long-term ecological behaviour of genetically engineered plants, we recommend strengthening the precautionary principle and prohibiting releases of genetically engineered organisms if

a. they can persist and invade the environment if they unintentionally escape their containment.

b. there are major doubts about whether they can be withdrawn from the environment within a reasonable period of time if this is urgently required.

c. it is already known that they will persist or show invasive behaviour after release into the environment.

Persistence and invasiveness are already crucial for risk assessment in the EU (EFSA, 2010). There is, however, a lack of clear regulatory criteria for dealing with genetically engineered organisms, where it is either unlikely or impossible to retrieve them from the environment. EFSA guidance (2010) does not foresee the rejection of applications for genetically engineered plants which are persistent and / or invasive. On the contrary, EFSA does not consider these biological characteristics to be a risk per se. A summary on p.49 of the guidance (EFSA 2010) states :

"The risk assessment should conclude on (1) the impact of the GM plant and/or hybridising relatives in the production system, particularly through increased weediness and more intense weed control; (2) the impact of the GM plant and/or hybridising relatives in semi-natural and natural habitats, through change in invasiveness or reduction of biodiversity or ecological function; (3) why any anticipated harm may be considered acceptable; and (iv) what risk management measures may be required to mitigate any harm."

In other words, if at the time of the application it is not already known whether the genetically engineered plant will actually cause serious harm to biodiversity or ecological function, then persistence and invasiveness can be classified as reasonable risks.

Testbiotech believes that this practice is not in line with the precautionary principle, which is paramount for risk assessment and risk management of genetically engineered organisms in the EU (Article 1, Dir. 2001/18). Genetically engineered organisms can be released or authorised on the basis of the precautionary principle even if there are still some uncertainties about actual risks for human health and / or the environment. After authorisation, they nevertheless still need to be monitored, and if evidence of adverse effects emerges, the products must be removed from the market. EU Directive 2001/18 foresees emergency measures in the event that new information on severe risks becomes available (Art 23):

> *"The Member State shall ensure that in the event of a severe risk, emergency measures, such as suspension or termination of the placing on the market, shall be applied, including information to the public. "*

Further, market authorisation must be reassessed after 10 years (Art. 15,4 of Directive 2001/18). Market authorisation can be terminated if there is new information on adverse impacts. Genetically engineered organisms must be removed from the market once they no longer have authorisation. (Art . 4 (5) of Dir. 2001/18).

The release of genetically engineered organisms that cannot be controlled in their spatio-temporal dispersal is in deep conflict with these provisions. The precautionary principle as established in Directive 2001/18 can only be implemented if efficient measures can be taken to remove the genetically engineered organism from the environment if this is urgently required. Therefore, spatio-temporal control is a prerequisite for implementing precaution. If a genetically engineered organism cannot be retrieved from the environment, the precautionary principle is meaningless. Consequently, spatio-temporal control is an obligatory precondition for any release of genetically engineered organisms.

As a legal dossier (Krämer 2013) commissioned by Testbiotech shows, the existing regulations in the EU do not provide sufficient legal clarity in this respect. For example, it is not clear, how to deal with genetically engineered organisms which are not fully characterised regarding their potential to persist and / or invade the environment or which are known to proliferate in an uncontrolled manner as soon as they escape beyond safety barriers as, for example, discussed in the case of genetically engineered salmon.

From the dossier of Krämer it also becomes evident that existing legislation in the US and under the Convention of Biological Diversity (CBD) the provisions are not sufficient to prevent the release of genetically engineered organisms that can not be retrieved.

7. Recommendations

In the countries and regions concerned, immediate measures should be taken to stop the further uncontrolled spread of genetically engineered plants into the environment as far as possible, and to stop the introduction of viable material that can be a source for renewed proliferation.

In the midterm, adequate regulations should be put in place to prevent new problems in this context and to strengthen the precautionary principle. On an international level, harmonised regulations should be put in place to prevent the centres of origin from becoming contaminated since they are of great importance for future breeding. This should be obligatory not only for the regulators in those countries and regions with centres of origin, but for all countries that want to make use of the relevant technologies.

On the basis of the documented cases and in the light of current uncertainties and lack of knowledge about dispersal, interactions with environment and the long-term ecological behaviour of genetically engineered plants, we recommend the prohibition of experimental releases, imports and commercialisation of genetically engineered organisms if

a. they can persist and invade the environment if they unintentionally escape their containment.

b. 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.

c. it is already known that they will persist or show invasive behaviour after release into the environment.

Several developments are making the need for such requirements even more urgent. Besides genetically engineered plants, genetically engineered insects have also been released[7]. Moreover, the first genetically engineered animals for food production are about to be commercialised with the genetically engineered salmon developed by Aquabounty[8]. Finally, it should not be overlooked that the development of new organisms with the help of synthetic biology will pose a new challenge for control and precaution (Then & Hamberger, 2010).

In the long term, it will be necessary to develop a concept to protect ecological and/or evolutionary integrity. Pimentel et al. (2000) define ecological integrity as follows:

> *"Ecological integrity should be defined as an ecosystem's undiminished ability to continue its natural path of evolution, its normal transition over time, and its successional recovery from perturbations."*

The aim of a concept of integrity would be to maintain functional biodiversity in the long term. To this Breckling (2009) adds the concept of "evolutionary integrity" which should be taken into account for the risk assessment of genetically engineered organisms:

> *"The concept of evolutionary integrity is relevant when transgenic DNA persists in gene pools of natural populations. It points to uncertainties how transgenes might interfere with the self-organisation of organisms and population dynamic implications."*

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7   See for example: http://www.genewatch.org/sub-566989

8   See for example: http://www.centerforfoodsafety.org/issues/309/ge-fish/about-ge-fish

Maintaining the ability of living beings to participate in further evolutionary processes through self-organisation and mutual adaptation can be understood as a crucial element in the protection of biological diversity. In the same way that we seek to protect eco-systems from long-lasting chemical substances that can accumulate in the environment[9], we can seek to protect ecological and evolutionary integrity by preventing the uncontrolled spread of synthetic and genetically engineered organisms in the environment (see also Then & Hamberger, 2010).

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9   See EU Regulation (EC) No 1907/2006

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