Transgene Escape: Genetically engineered oilseed rape out of control - a global perspective

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Content

Summary

Genetically engineered plants were created 30 years ago and have been grown commercially for 20 years. Throughout this time transgenes have escaped from fields and containments in several regions (see for example Ellstrand, 2012). This is especially true for genetically engineered oilseed rape (canola).

Our report provides a global overview of the uncontrolled spread of genetically engineered oilseed rape in various regions of the world. We found publications from Canada, the US, Japan, Australia, Switzerland and the European Union. Countries such as Canada and Japan are of particular concern since we have to assume that there has already been some geneflow of transgenes into populations of wild relatives in these countries.

Apart from commercial cultivation (such as that in Canada and the US) and experimental field trials as for example in Germany it is the import and transport of viable grains for food and feed production (such as EU and Japan) that are the source of uncontrolled dispersal of these plants.

In the EU genetically engineered oilseed rape was never grown commercially. Nevertheless, contaminations with transgenic oilseed rape produced by Bayer are still found regularly despite market authorisation for this oilseed rape being withdrawn in 2007.

It is quite difficult to make reliable predictions which genetically engineered plants will persist or become invasive and what their long term environmental impact will be. There are too many factors such as ongoing climate change which can have a major impact on the invasive potential of some plant species and their ecological behaviour.

This report calls for immediate and comprehensive regulation and for political activities to strengthen the precautionary principle. The release of genetically engineered organisms should not be allowed if they cannot be retrieved from the environment if this is urgently required.

Oliseed rape growing wild close to field margin with flowering plants on a field in the back (Foto: G. Menzel)

1. Case Study: Canada

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). Several other studies which were 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 nearly no fitness costs are associated with the stacking of transgenes in oilseed rape plants (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 93 out of 100 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).

2. Case Study: USA

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 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.

3. Case Study: Japan

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 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 feral 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 feral 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 plants with greater height 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. Case Study: Australia

Genetically engineered oilseed rape has been cultivated in western Australia since 2009. Western Australia is a so-called GMO free region, and thus 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 on 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. Weaknesses of oversight stated by CCWA (2012) comprise the nonexistence of monitoring programmes and missing regulations regarding ruderal transgenic populations.

5. Case Study: European Union

In Germany (as well aus in many other European countries), many 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);
- implementation of requirements by the companies was not sufficient (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 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 with low level contaminations a long period of time is needed to minimise the uncontrolled dispersal of genetically engineered oil seed 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.

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 technically 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/EC.

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.

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

6. Case Study: 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 from imports is remarkable because since 2008, import of transgenic oilseed rape is prohibited in Switzerland. Only small traces of not more 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 were identifying hot spots of transgenic plants at places were unloading takes place.

About the biology of oilseed rape

Family:

Brassicaceae

Centre of origin:

Oilseed rape stems from natural crossing of B*. oleracea a*nd *B. rapa* which likely happened in Mediterranean or Atlantic area of Europe less than 10.00 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:

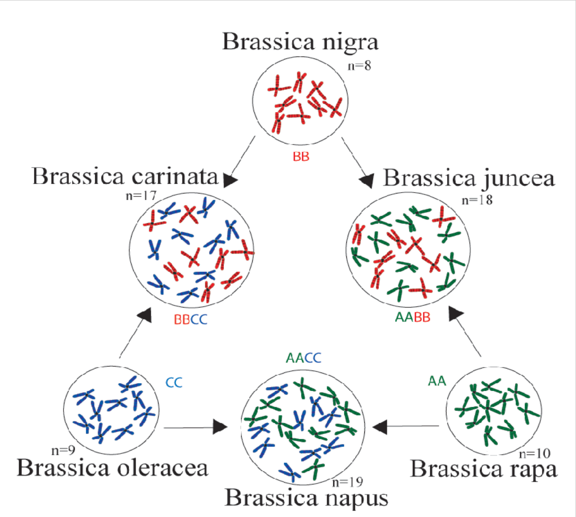
4-5 days under natural conditions (OECD, 2012)

Seed persistence/dormancy:

empirical data up to now more than 11 years Lutman et al. (2003) with 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).[1]



1 http://en.wikipedia.org/wiki/Triangle_of_U

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
Raphanus raphanistrum
Sinapis alba
Sinapis arvensis

Weediness / invasiveness:

All cultivated *Brassica* species are also weeds. Oilseed rape can appear in feral ruderal populations along field edges and roadsides, Feral populations are able to sustain themselves in a half permanent form Pivard et al. (2008). According to Munier et al. (2012), herbicide tolerant oilseed rape is considered as weed.

Wild relatives with potential for invasiveness/weediness:

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

Possible transgene-mediated fitness advantage:

Hybrids between *B. napus a*nd *B. juncea* (Di et al., 2009) as well as *B. napus* and *B. rapa* (Rose et al., 2009) showed only slight fitness costs compared with the wild species. Tests with Bt oilseed rape showed that the plants have fitness advantages under pest insect pressure (Mason et al., 2003). Also herbicide tolerance will give advantages for relevant transgenes if plants are sprayed. According to Claessen et al. (2005) also transgenic modifications for modified oil content (like higher content of stereat or laurat) leads to fitness advantages in oilseed rape. Related species like *B. rapa* und *Raphanus sativus* also acquire higher fitness in case of introgression of Bt genes (Letourneau & Hacker, 2012). According to simulations by Meier et al. (2013), this might also be the case for *Raphanus raphanistrum.*

Discussion

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[2] 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;*
> *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. This is especially relevant with regard to current climate change. According to many experts, ongoing climate change will lead to considerable changes in the communities of wild species. Plants and animals with invasive potential might occupy new ecological niches. For example some experts (Clements & Ditommaso, 2011) expect climate change to cause an exponential growth in populations of invasive plant species.

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 &

[2] GMLS Konferenz in Bremen, 2012, http://www.gmls.eu/

Weis, 2009). Their study shows there was a change in the time for flowering resulting in matching of flowering between species.

Recommendations

In the countries and regions concerned, immediate measures should be taken to stop 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 being contaminated since they are of great importance for future breeding. This is a duty not only for the regulators in those countries and regions but of all countries that want to make use of the relevant technologies.

On the basis of the documented cases and in the light of current boundaries in the knowledge of dispersal, interactions with environment and ecological behaviour, 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.

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