Transgene flow: Facts, speculations and possible countermeasures

Gerhart U Ryffel*
Institut für Zellbiologie (Tumorfororschung); Universitätsklinikum Essen; Essen, Germany

Keywords: apomixis, cisgenesis, cleistogamy, infertility, intragenesis, transgene flow, transgenic mitigation
Abbreviations: EPSP, enolpyruvylshikimate-3 phosphatase conferring glyphosate resistance; PAT, phosphinothricin acetyl transferase conferring glufosinate resistance; VT, volunteer

Gene flow is an inherent feature in all living organisms allowing the transmittance of genes to the next generations and forming the basis of evolution. Usually gene flow is restricted to a given species or to closely related species that are sexually compatible. In very rare cases genes can also be transferred between species that cannot mate and this is referred to as horizontal gene transfer.

With genetic engineering it is possible to introduce any gene into a genome. The introduced gene is either a cisgene, if the manipulated gene is derived from the same species or a sexually compatible species, or a transgene, if the gene is derived from a species that is not compatible with the host. In the case of a transgene a novel gene is introduced into a given genome and this may constitute some concern, as this transgene can be transmitted to other organisms. Thus, the transgenic organism has not only novel traits whose consequences should be carefully evaluated, but also the potential to transmit this property to any sexually compatible plant. Recognizing this potential transgene flow, cultivation of GM plants for research and commercial purposes have been performed under some restriction to avoid the escape of a transgene. After 20 y of commercial GM crops we realize that we failed to contain transgenes in a controlled manner.

Unintended Transgene Escape

This present article reviews data reporting on transgene flow. The focus is on events where a transgene has escaped control unintentionally and outside of its primary agricultural productive fields. These findings are not too surprising, as GM plants can usually grow and reproduce as the plants they are derived from. The extent of escape depends on the properties of the GM plant itself, but is also highly affected by the strategies used to limit uncontrolled escape. Thus, for a given plant it is important whether reproduction involves self or cross pollination, whether the pollen are efficiently dispersed by insects or by wind as well as whether the seeds are scattered and persist over a long time period. Depending on these features adapted techniques have been established to limit these natural processes of reproduction. They include isolation distances between GM and corresponding conventional plants to avoid cross-fertilization. Importantly, inadequate handling such as seed lot contamination, mixing during sowing, harvest and trade can also lead to transgene escape. Such events have made headlines in the media, but in most cases limited information has been published in peer reviewed journals (for a review see reference 5). Concerning unintended escape it is necessary to distinguish between volunteerism of the original GM crop, intraspecific hybridization with variants or the wild type plant and interspecific hybridization with sexually compatible species (Fig. 1).

In most cases the unintended escape of volunteers occurs within agricultural fields, where either the seed lots of conventional crops are mixed with GM seeds or seeds from GM crops germinate within the next season’s crop, as was the case for...
instance with GM maize containing a veterinary vaccine in the field of next year’s soybean crop. Transgene escape may not only occur in area within or bordering the GM agricultural fields, but may also happen far away such as in the case of GM oilseed rape in Japan (Table 1) and in Switzerland. 7,8 In both cases GM oilseed rape is not cultivated in these countries, but has been grown in North America and the grains transported for processing into both animal feed and human food have been lost on its way. These examples illustrate the global dimension of the problem, as regions far away may be affected.

Since GM plants or its volunteers grow frequently in vicinity of variants, hybridization with these plants has been observed as expected. Therefore, a precautionary approach seems appropriate and in fact in many cases GM crops are not grown in the vicinity of non-GM crops. The ongoing discussion on the extent of transgene escape in maize illustrates also the problem to quantify transgene flow accurately and to eliminate transgenes that may have escaped.

Mexico, where maize landraces contain various transgenes (Table 1), although GM corn is not allowed for cultivation in this country. Initially these reports were highly controversial, but reviewing the studies there is good evidence for the escape of transgenic traits into maize (Table 1). As Mexico is the center of crop origin and diversity of maize, some people are concerned that the gene pool could be contaminated irreversibly. There is not much dispute that the various landraces of maize and teosinte have coexisted over centuries without losing their identities, although there is an extensive gene flow between the species.16 However, we cannot ignore that the presence of a transgene such as the Bt gene derived from bacteria constitutes a novel trait whose effect we cannot predict upon introgression into the teosinte population. Therefore, a precautionary approach seems appropriate and in fact in many cases GM crops are not grown in the center of crop origin and diversity. The ongoing discussion on the extent of transgene escape in maize illustrates also the problem to quantify transgene flow accurately and to eliminate transgenes that may have escaped.

A similar discussion about the integrity of a plant’s genome in its center of origin has been started, when several transgenes were detected in wild cotton 15 y after the beginning of GM cotton cultivation in Mexico.17 Looking for recombinant proteins by ELISA, expression of four transgenic traits was detected. Since the combinations of these recombinant proteins differ among the four contaminated metapopulations and the haplotype differs to the GM cultivars, it seems likely that each combination reflects independent and multiple transgene flow events. It also appears that recombination of various transgenic traits has occurred in
### Table 1. Unintended escape of transgenes

| Species                  | Region          | Escaped transgene | VT<sup>(a)</sup> | Hybridization | Comment and References                                                                 |
|--------------------------|-----------------|-------------------|-------------------|---------------|---------------------------------------------------------------------------------------|
| **Oilseed rape**<br> *Brassica napus* | Japan <sup>(b)</sup> | EPSP              | +                 |               |                                                       |
|                          | Japan <sup>(b)</sup> | EPSP, PAT         | +                 | +             | Origin of double resistance unclear                                                   |
|                          | Japan <sup>(b)</sup> | EPSP, PAT         | +                 | +             |                                                       |
|                          | Japan <sup>(b)</sup> | EPSP, PAT         | +                 | +             |                                                       |
|                          | Japan <sup>(b)</sup> | EPSP, PAT         | +                 | +             |                                                       |
|                          | Japan <sup>(b)</sup> | EPSP, PAT         | +                 | +             |                                                       |
|                          | Switzerland <sup>(c)</sup> | EPSP              | +                 |               |                                                       |
|                          | Switzerland <sup>(c)</sup> | EPSP              | +                 |               |                                                       |
|                          | Alberta, Canada | EPSP              | +                 |               | Neighboring field, multiple herbicide resistance                                        |
|                          | Saskatchewan, Canada | EPSP, PAT | +                 |               | Neighboring field, multiple herbicide resistance, double resistance in seedlots         |
|                          | Western Canada   | EPSP, PAT         | +                 |               | Double-resistant seedlots                                                               |
|                          | Manitoba, Canada | EPSP, PAT         | +                 |               | Double resistance by transgene flow in escaped populations                              |
|                          | North Dakota, USA | EPSP, PAT        | +                 |               | Double resistance                                                                      |
|                          | Canada?          | PAT               | Brassica rapa     |               |                                                       |
|                          | Vancouver, Canada | EPSP              | Brassica rapa     |               |                                                       |
|                          | Québec, Canada   | EPSP              | Brassica rapa     |               | Commercial fields, no escape to Raphanus raphanistrum, Sinapsis arvensis or Erucastrum gallicum |
|                          | Québec, Canada   | EPSP, PAT         | Brassica rapa     |               |                                                       |
|                          | Québec, Canada   | EPSP              | Brassica rapa     |               | Persistence over 6 y                                                                    |
| **Maize**<br> *Zea mays* | Mexico          | Vector, Cry1Ab    | +                 |               |                                                       |
|                          | Mexico          | Cry1Ab/Ac, EPSP   | +                 |               |                                                       |
|                          | Mexico          | Not given         | +                 |               | Reviews and includes unpublished data                                                  |
|                          | Mexico          | Vector           | +                 |               | Controversy in refs 12; 13                                                              |
|                          | Mexico          | Cry1Ab/Ac, EPSP   | +                 |               |                                                       |
| **Cotton**<br> *Gossypium hirsutum* | Mexico | Cry1AbAc, Cry2Ac, EPSP, PAT | + | |                                                       |

<sup>(a)</sup> VT = Vector Transgene

(continued on next page)
the field, as some of the cotton plants contain stacked traits that are not commercially available. As cotton is primarily self-polli-
nated, transgene flow probably has occurred mostly via seed and secondary cross-pollination events. This assumption is supported by the fact that cotton seeds from GM plants are transported as livestock feed over long distances, thus possibly explaining that some escaped transgenes were found 755 km away from an approved GM cotton plot. So far, these data in cotton have not been verified in independent studies and an analysis on the DNA level is needed to get full acceptance in the scientific community.

Beside transgene flow within variants and wild type plants, hybridization with related wild species has been reported in sev-
eral cases (Fig. 1). Since the oilseed rape cultivar (*Brassica napus*, N = 19, AC), a human selected hybrid between *Brassica oleracea* (N = 9, C) and *Brassica rapa* (N = 10, A) readily hybridizes with the weedy relative *Brassica rapa*, it was no surprise to find in field experiments transgenic glufosinate resistant interspecific hybrids that were fertile and upon backcrossing with *Brassica rapa* resulted in weed-like herbicide resistant plants. Such a rapid spread of transgenic traits of oilseed rape to weedy relatives has been confirmed subsequently in many studies worldwide (Table 1).

Another illustrative example of transgene flow has been documented after experimental planting of glyphosate resistant creeping bentgrass, a common turfgrass used in golf courses (Table 1, reviewed in). A few km away from the test plot in Oregon feral transgenic creeping bentgrass was found in subsequent years, reflecting the high potential of creeping bentgrass for gene escape. More significantly, a transgenic interspecific hybrid was identified and based on molecular marker analysis it was deduced the hybrid was produced on a feral transgenic bentgrass that received pollen from rabbitfoot grass.

The abundant detection of escaped transgenes (Table 1) is of some immediate concern, as it may endanger the co-existence measures that should permit consumer choice and freedom of agricultural production. Furthermore, as the unintended escape of transgenes is a major cause of public distrust in GM crops, we should improve our containment strategies. More importantly, the frequent unintended presence of stacked transgenes, especially herbicide resistance traits, may have negative effects for the farmers, as it interferes with a predictable management of the cultivated crops. Although transgene flow as such may have not an adverse environmental effect, the long-term consequences for species conservation and biodiversity might be relevant. At the present time it is hard to predict the long-run consequences of transgene flow, as the escape events seen may not necessarily lead to stable introgression of the transgene into variants, weedy relatives or wild plants. In addition, we cannot predict whether the introgressed transgene leads to increased invasiveness (super-weeds), to a permanent gene pool modification or even to the extinction of a wild species. Some specific warning could be deduced from a recently observed interspecific gene transfer from a glyphosate-resistant Palmer amaranth to a spiny Amaranth, although the transferred EPSPS amplicon has been selected by glyphosate selection and is not a transgenic trait. This gene transfer is of concern, as it shows the spread of herbicide resistance between different species, both of which are noxious weeds. This example also illustrates that negative effects of gene flow are not restricted to GM crops.

The outcome of transgene escape will highly depend on the ability of the transgene to influence the fitness of the plant containing the introgressed gene. Although several studies have been made to address these questions, a definitive answers cannot be given, as we hardly understand the complexity of the ecosystem. But it seems inappropriate to wait until for instance we have full proof that transgene flow has irreversibly destroyed the gene pool of a plant in its center of origin and diversity. In addition, we have to consider that an unlikely negative outcome may get quite likely, if many different GM crops are cultivated on a large scale all over the world. The numbers given in Table 2

---

**Table 1. Unintended escape of transgenes (Continued)**

| Species                  | Region       | Escaped transgene | VT<sup>(a)</sup> | Hybridization | Comment and References |
|--------------------------|--------------|-------------------|-------------------|---------------|------------------------|
| Creeping bentgrass       | Oregon, USA  | EPSP              | +                 | Intraspecific  | Nonagronomic habitat following test production<sup>(d)</sup> |
| *Agrostis stolonifera*   |              |                   |                   | wild          |                        |
|                          |              |                   |                   | Inter-specific|                        |
| Oregon, USA              | EPSP         |                   | +                 |               | Establishment and persistence after 3 y<sup>(e)</sup> |
| Oregon, USA              | EPSP         | Rabbit footgrass  |                   |               | Transgene transferred through maternal lineage<sup>(22)</sup> |
|                          |              | *Polypogon monspeliensis* |               |               |                        |

<sup>(a)</sup> Volunteers are only listed, if found far away from the production fields. Oilseed volunteers in countries where commercially grown are not included.

<sup>(b)</sup> Not grown in Japan.

<sup>(c)</sup> Not grown in Europe.

<sup>(d)</sup> A follow-up study of the pollen-mediated, landscape-level transgene flow from a 162 hectares production test area.

Comparable listings have been published recently.<sup>(99,100)</sup>
try to illustrate this problem by using various assumptions whose values are only rough estimates. However, it illustrates that an unlikely event gets likely, if the event occurs many time. We are well aware of this principle, as we all know that it is very unlikely to get killed oneself in a car accident, but that many people are killed every day worldwide. Therefore, the ultimate goal should be to minimize transgene escape into the wild as efficiently as possible using all techniques available. As discussed above for cotton and maize the potential influence of GM crop outcrossing in the center of origin deserves special attention. A similar controversy has been recently initiated for brinjal (eggplant) where the lack of adequate information on the interfertility relationship of brinjal and its wild, weedy and cultivated relatives has been deplored.29 To take care of these uncertainties I propose that we design and use GM crops in a more sophisticated way. Some approaches are quite straight forward with the technologies available, whereas others will require major research efforts. Any strategy that lowers the probability of transgene escape should be considered but also used, if we agree upon a sustainable agriculture that is acceptable to the public as much as possible. A major problem will be to balance the efforts taken with the potential benefit of a specific GM crop. Clearly, a GM ornamental plant with a fancy new color has a lower beneficial potential than a biofortified GM crop needed for better nutrition in developing countries.

Counter Measurements

To avoid transgene escape different strategies are possible that should take care of the probability that a given transgene escapes from a specific GM plant. A useful assessment has been made by dividing the crops into four risk categories for the introgression of transgenes: very low risk, low risk, moderate risk and high risk.30 According to this categorization that reflects the probability of transgene escape soybean belongs to the very low risk crops and indeed no unintended escape has been reported, although GM soybean are one of the mostly grown GM crops. In contrast, maize and cotton belong to the low risk crops and escape has been found (Table 1). Correspondingly in oilseeds rapeseed considered to be a moderate risk crop, escaped transgenes have frequently been documented (Table 1).

Furthermore, the counter measurements used should take into account the trait conferred by the transgene. Obviously, the likelihood that a transgenic property confers a fitness advantage for an escaped transgene should guide the type of precautionary strategy. Any herbicide resistance gene has a high probability to be transmitted to related weeds that are present in the agricultural fields treated with the corresponding herbicide. The surprising finding, that a glyphosate resistance gene in weedy rice conferred fitness benefit in the absence of herbicide documents that pleiotropic effects of transgenes should not be ignored.31 However, this finding has been disputed32-35 and the discussants would only agree that the transgenic weedy rice has an enhanced fecundity whose origin could also reflect an insertional event. In the case of Bt toxin a selective advantage may be given, if the transgene escapes into wild type plants, although selection pressure may be lower compared to herbicide resistance. An often cited example of fitness benefit has been reported in transgenic wild sunflowers.36 As expected the Cry1Ac gene reduces herbivory in sunflowers, but in addition enhances seed production. Although the cause of the observed increase in fecundity is unclear, the example illustrates that a careful analysis is required. Reviewing several experiments it is appropriate to consider the likely effects of various transgenes on a case-by-case basis.37 This evaluation will get more important when GM crops are planted that affect directly important ecological parameters such as resistance to major abiotic stress, including drought, salt and high temperature.27

Risk assessment of transgene flow is an important issue in all cases of commercial GM crops and includes potential loss in abundance or diversity of valued flora and fauna. This most challenging undertaking is exemplified in a detailed analysis of biofortified sorghum to be introduced in Africa.38 A major problem in this kind of analysis is the agreement on the plausibility of specific harms. Furthermore, risk assessment should also include the consequences of recombination of disparate transgenes, a process that has frequently been observed with escaped transgenes (Table 1). Realizing that risk assessment cannot predict with 100% certainty the extent of transgene escapes,39 a cautionary release of GM crops is started usually to monitor potential harmful effects on compatible relatives. To prevent such a labor intensive investigation and to avoid decision that are to some extent subjective, elimination of transgene flow seems to be a most valid option.

In the following section I list the most important counter measures against transgene flow and discuss their pros and cons.
Careful processing of the GM crops

Process seeds at the agricultural production site

As best exemplified by oilseed rape the worldwide trade of the seeds is a major cause to disseminate GM plants far away from the production site. Therefore, a strict regulation to process seeds at the site of production is an appropriate approach to avoid unintended scattering of the seeds during transportation. This strategy could be started without delay for all the GM crops grown today where seeds are transported and especially where escape has been documented (Table 1), i.e. oilseed rape, maize and cotton.

Careful selection of the recipient plant for GM

Infertility of the plant

Several crop cultivars such as cassava, banana and potatoes are sterile. Provided this sterility is not leaky, these cultivars should be preferably used for genetic engineering. Male sterile plants are present in more than 600 plants and in other plants it is possible to induce male sterility by gene manipulation. Beside nuclear encoded male sterility cytoplasmic male sterility (CMS) would be an alternative. In this case maternally inherited mitochondrial genes confer pollen sterile plants. However, gene transfer to the nucleus, which results in termination in maternal inheritance, cannot be excluded and fertility can also be restored by nuclear gene expression. Clearly, male sterility should be combined with female sterility to get reliable containment of the transgenic trait.

Cleistogamy, the trait of non-opening flowers

A reduced transgene flow can also be obtained by cleistogamy found in some crops species such as barley, soybean and rice. A recent analysis claimed an almost complete suppression of crossing in a cleistogamous rice line, but the number of xenia seeds analyzed is too low to allow accurate quantification. It should also not be ignored that in some crops the lack of pollen may have a negative impact on pollen feeding insects.

Apomixis, i.e. clonal production of seeds

In crops where the seeds are harvested transgene flow could be avoided by using apomictic traits that are present in more than 400 plant species. In these plants seeds are clones without sexual reproduction and thus develop without fertilization (parthenogenetically). In combination with male sterility these plants offer a convenient way to contain a transgene within the GM crop. Whereas some cultivars of a few crop plants such as cassava, oranges and sugar beet are apomictic, most other plants do not show this asexual process of reproduction. Although several genes and mechanisms involved in apomixis have been identified, substantial efforts are still needed to engineer apomixis into sexual crops. It is not yet clear, whether apomictic plants can be generated to ensure a complete containment, since the naturally occurring apomictic bahiagrass (Paspalum notatum) transferred transgenic herbicide resistance at about 0.2%. The use of apomixis in cropping plants would be most welcome not only to get a reliable containment strategy, but also to simplify the production of hybrids that could be propagated indefinitely without losing the benefits of heterosis. Apomictic plants would also allow the use of seeds in subsequent seasons, a fact that may be welcome by farmers, but a problem for the companies selling these products.

Improved techniques in generating GM plants

Site-directed mutagenesis

In the last few years techniques have been established to mutate more or less any site in the genome by using designer nucleases (zinc finger nucleases, TALEN or CRISPR-Cas). Such site directed mutagenesis could replace in many cases the insertion of an expression cassette for RNA interference that is intended to knock-down the expression of an endogenous gene. Using site-directed mutagenesis the introduction of foreign DNA can be avoided, the new trait is comparable to a natural mutation and the concern of transgene escape is no issue. In cases where some residual gene activity is essential for plant growth, alternative mutations could be designed. For instance the mutation could be targeted to the protein coding sequence to down-regulate the stability of the gene product or to regulatory sites of the gene to lower the transcription. Such hypomorphic mutations are frequently seen in mutations selected by domestication.

Cisgenesis and intragenesis

If possible genetic engineering should transfer genes from sexually compatible species. This strategy is referred to as cisgenesis, if only a single continuous DNA fragment is inserted or intragenesis, if multiple linked DNA fragments from sexually compatible species are used. A critical distinction between these two novel approaches of gene manipulation is that the phenotype of cisgenic plants could be obtained by classical breeding, whereas intragenesis can yield novel phenotypes beyond the reach of breeding. Since cisgenesis does not modify the natural gene pool, similar hazards can be associated with cisgenic and conventionally bred plants. In addition, cisgenesis/intragenesis combined with site directed gene insertion seems to be superior to classical breeding, as the site of gene insertion is predictable and linkage drag is avoided. Therefore, this improved technology of gene insertion should be mandatory nowadays and the classical Agrobacterium mediated transformation should be abandoned.

Chloroplast engineering

As chloroplasts are primarily maternally inherited and thus pollen-mediated transgene dispersal is avoided, this approach has been proposed for genetic engineering. However, the success of this containment strategy is limited in some plants, as biparental inheritance of plastids is well documented and transfer of
transgene from the chloroplast to nucleus is known.\textsuperscript{60} It is also possible that the transgene can get out of control by fertilization of the transgenic plant by wild type pollen, as documented in the case of creeping bentgrass.\textsuperscript{22} Therefore, chloroplast engineering should be complemented by female sterility.

Disabling transgene escape

Transgene removal from pollen

In this approach the transgene insert is flanked by specific sequences that allow pollen specific excision of the transgene by a site-specific recombinase encoded within the transgenic construct (reviewed in reference 44). With this approach normal production of pollen would be assured. However, the efficiency of excision by recombinases as well as by site-specific nucleases is inherently quite low; with a 99\% performance considered to be excellent.\textsuperscript{61} Therefore, the performance of this strategy, that involves a lot of experimental work and has been tested mainly in model system, is at the moment too low to reduce transgene escape. It seems rather to be a good choice to remove selectable marker genes used to obtain transgenic plants.\textsuperscript{62} In addition, successful removal of transgenes in pollen would preclude seed collection by farmers for subsequent years.

Gene use restriction technology (GURT)

This strategy has primarily been designed to restrict access to genetic materials and their associated phenotypic traits.\textsuperscript{63-65} The primary goal was to generate GM crops that maintain intellectual property protection for the industry (terminator technology) by controlling seed production. The various approaches envisaged are based on a complex signaling cascade to either eliminate the entire plant genome or to remove selectively the transgene from the genome. So far no system has been established and tested to prove its ability to inhibit transgene flow. In the light of the public discussion that this technology limits farmer’s access to novel traits and further opens the door to a dominance of industry over agriculture, it will require most convincing data proving that the technique is a secure way to inhibit transgene flow efficiently before any commercial application can be envisaged.

A second transgene to prevent spread of transgenic traits

This concept of transgenic mitigation (TM) has initially been proposed in 1999 to decrease fitness in volunteers and hybrid progeny by coupling the desired transgene in tandem with a TM gene that would render a fitness disadvantage to the transgenic recipient.\textsuperscript{64,66} Experiments in Brassica show that the mitigation technology works efficiently in this crop weed system, but so far field studies have not validated its utility to minimize gene flow.\textsuperscript{67} However, such an approach with a tandem transgenic construct would only operate as long as the TM gene stays linked to the transgenic construct. To lower the probability to lose the TM trait two flanking TM genes could be used.\textsuperscript{66} As a variation the transgenic construct has been supplemented by an RNA interference cassette inactivating a detoxification enzyme thus allowing the killing of a transgenic plant by herbicide application in rice\textsuperscript{68,69} or corn fields.\textsuperscript{70} The herbicides selected are already used in commercial production fields. Thus, killing of escaped transgenes within the cultivated area may be acceptable, but its use outside of the agricultural area may be problematic.

Concluding Remarks

The available data clearly show that unintended transgene escape occurs with at least three commercial GM crops (Table 1) and therefore counter measurements should be taken as soon as possible. Obviously, an easy and first step could be the processing of grain of GM crops at the production site to avoid transport loss of seeds that germinate out of control. This would eliminate many observed cases of escape and would help to regain trust into the use of GM plants. In cases where GM plants are not yet planted commercially it would be reasonable to review the potential of transgene flow more critically. GM rice and GM wheat are definitely two crops where transgene flow plays a major problem, especially because the transgene can readily be transferred within the cultivated area to weedy relatives. In cultivated rice fields wild rice (red rice) is a most common weed that evolved multiple times and in several different ways.\textsuperscript{71} As illustrated with non-transgenic imidazolinone-resistant Clearfield\textsuperscript{®} rice, herbicide resistance was found within a few years in wild rice when Clearfield\textsuperscript{®} rice was cultivated in the USA, Brazil, Colombia, Costa Rica, Uruguay and Italy.\textsuperscript{72} This gene flow within the crop to the red rice is the starting point of escape\textsuperscript{73,74} that should be avoided right at the beginning in any commercial GM rice production field. Similarly, wheat fields are frequently infested by the sexually compatible jointed goatgrass (Aegilops cylindrical) and the transfer of the non-transgenic imidazolinone-resistance has been observed under natural field conditions.\textsuperscript{75,76} A comparable situation concerns sugar beet where herbicide tolerant GM plants have been grown commercially since 2009,\textsuperscript{77} although wild beet species may be present within sugar beet fields and transgene flow is almost unavoidable.\textsuperscript{78} The ban of GM sugar beet planting in regions with high chance of introgression is only second choice compared to a beet modified in such a way to exclude gene flow. In all three examples with an herbicide resistance gene the survival of the escape transgene is selectively favored by the use of the herbicide for weed control. Such a selection should not be done without some efficient counter measures to avoid transgene escape.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
References

Telem RS, Wani SH, Singh NB, Nandani R, Sadhukhan R, Bhatthacharya S, Mandal N. Cisgenics - a sustainable approach for crop improvement. Curr Genomics 2013; 14:468-76; PMID: 24396278; http://dx.doi.org/10.2174/1389470910039013

Holme IB, Wendt T, Holm PB. Intragenesis and cisgenesis as alternatives to transgenic crop development. Plant Biotechnol J 2013; 11:395-407; PMID: 23421562; http://dx.doi.org/10.1111/j.1365-202x.12059.x

Mallory-Smith C, Zapido M. Gene flow from glyphosate-resistant crops. Pest Manag Sci 2008; 64:428-40; PMID: 18181145; http://dx.doi.org/10.1002/ps.1517

Devo Y, Dillon K, Demont M. How can flexibility be integrated into coexistence regulations? A review. J Soc Food Agric 2014; 94:381-7; PMID: 23957654; http://dx.doi.org/10.1002/jfda.6358

Demeke T, Perry DJ, Scoworth CR. Adventitious presence of GMOs: scientific overview for Canadian grains. Can J Plant Sci 2006; 86:1-23; http://dx.doi.org/10.4141/cjps06075-114

Spik A, Tysmans RM, Fischer R, Ma JK, Sparow PA. Evolution of a regulatory framework for pharma-aceuticals derived from genetically modified plants. Trends Biotechnol 2008; 26:506-17; PMID: 18676047; http://dx.doi.org/10.1016/j.tibtech.2008.05.007

Schonenberger N, D’Andrea L. Surveying the occurrence of spontaneous glyphosate-tolerant genetically engineered Brassica napus L. (Brassicaeae) along Swiss railways. Environ Sci Europe 2012; 24:23; http://www.enviroeurope.com/content/24/123

Hecht M, Oehn B, Schulze J, Broidmann P, Bagur C. Detection of feral GFT73 transgenic oilseed rape (Brassica napus) along railway lines on entry routes to oilseed factories in Switzerland. Environ Sci Pollut Res 2014; 21:1455-65; PMID: 23971737; http://dx.doi.org/10.1007/s11356-013-1881-9

Hall L, Topinka K, Huffman J, Davis L, Good A. Pollen flow between herbicide-resistant Brassica napus is the cause of multiple-resistant B-napus vol-unteers. Weed Sci 2000; 48:688-94; WOS:000166182800008; http://dx.doi.org/10.1614/0043-1745(2000)048[0688:FACREM]2.0.CO;2

Mallory-Smith CA, Sanchez Olguin E. Gene flow from genetically modified crops to their wild relatives. Nat Rev Genet 2003; 4:806-17; PMID: 14526376; http://dx.doi.org/10.1038/nrg1179

Wang W, Xia H, Yang X, Xia T, Si HJ, Cai XX, Wang F, Su J, Snow AA, Lu BR. A novel 5-enolpyr-uvoylshikimate-3-phosphate (EPSP) synthase trans- gene for glyphosate resistance stimulates growth and fecundity in weedy rice (Oryza sativa) without her-bicide. New Phytol 2013; 202:679-88; PMID: 23996567; http://dx.doi.org/10.1111/nph.12428

Gressel J, Neal Stewart C Jr, Gidding LV, Fischer AJ, Streibig JC, Burgos NR, Trewavas A, Merotto A Jr, Leaver CJ, Ammann K, et al. Overexpression of eps transgene in weedy rice: insufficient evidence to support speculations about bioassay. New Phytol 2014; 202:1899-1904; PMID: 24657822; http://dx.doi.org/10.1111/nph.12615

Grunevald W, Bury J. Comment on ‘A novel 5-enolpyruvoylshikimate-3-phosphate (EPSP) syn- thase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (Oryza sativa) without herbicide’ by Wang et al. (2014). New Phytol 2014; 202:367-69; PMID: 24654784; http://dx.doi.org/10.1111/nph.12683

Lu BR, Snow AA, Yang X, Wang W. Scientific data published by a peer-reviewed journal should be properly interpreted: a reply to the letter by Gressel et al. (2014). New Phytol 2014; 202:363-66; PMID: 24657833; http://dx.doi.org/10.1111/nph.12684

Snow AA, Pilon D, Riesberg LH, Paulsen MJ, Pleseck N, Reagan MR, Wolf DE, Selmo S, A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. Ecol Appl 2003; 13:279-86; PMID: 12055088; http://dx.doi.org/10.1890/0012-9623(2003)013[0279:ABTRHA]2.0.CO;2

Chapman MA, Burke JM. Letting the gene out of the bottle: the population genetics of genetically modified crops. New Phytol 2006; 170:429-43; PMID: 16626666; http://dx.doi.org/10.1111/j.1469-8137.2006.01710.x

Hokanson KE, Ellerton NC, Ouedraogo JT, Olweny PA, Schaal BA, Raybould AF. Biofortified sorghum in Africa: using problem formulation to inform risk assessment. Nat Biotechnol 2010; 28:908-3; PMID: 20829822; http://dx.doi.org/10.1038/nbt.1810

de Jong TJ, Rong J. Crop to wild gene flow: Does more sophisticated research provide better risk assessment? Environmental Science & Policy 2013; 27:135-40; WOS:000316843500012; http://dx.doi.org/10.1016/j.envsci.2012.12.002

Sayre R, Beeching JR, Caloone EB, Egovi C, Faustegui C, Felman J, Fregene M, Grussem W, Mallowa S, Manany M, et al. The BioCassava plus program: biofortification of cassava for sub-Saharan Africa. Annu Rev Plant Biol 2011; 62:251-72; http://dx.doi.org/10.1146/annurev-arplant-042110-103751

Tripathi L. Biotechnology in Musa Improvement. In: Banana Breeding Progress and Challenges, Pillay M, Tenkuana A Eds, CRC Press; 2011:19-36; http://dx.doi.org/10.1201/b10154-12

Celic S, Scurrall M, Cowgill S, Chumbiana S, Green J, Franco J, Main G, Kiezebrink D, Visser RG, Arkinson HJ. Environmental bioassay and transgenic potato in a centre of diversity for this crop. Nature 2004; 432:222-25; PMID: 15538870; http://dx.doi.org/10.1038/nature03048
43. Chen L, Liu YG. Male sterility and fertility restoration in crops. Annu Rev Plant Biol 2014; 65:579-606; PMID: 24313845; http://dx.doi.org/10.1146/annurev-plant-050213-041119.

44. Moore GA. Oranges and lemons: clues to the taxonomy and introgression of Citrus species. Trends in Biotechnology 2010; 28:3-8; PMID: 20128846; http://dx.doi.org/10.1016/j.tibtech.2009.09.008.

45. Hohne S, Proscher S, Schiemann J. Evaluating biocultural containment strategies for pollen-mediated gene flow. Environ Biosafety Res 2010; 9:67-73; PMID: 22138801; http://dx.doi.org/10.1007/s00425-013-2019-5.

46. Ohmori S, Tabuchi H, Yatao O, Yoshida H. Agromics and traits and gene containment capability of cleistogamous rice lines with the superwoman1-cleistogamy mutation. Breed Sci 2012; 62:124-32; PMID: 23163523; http://dx.doi.org/10.1270/jsbbs.62.124.

47. Spillane C, Curtis MD, Groskniall U. Apomixis technology development-virgin births in farmers' fields? Nat Biotechnology 2004; 22:687-91; PMID: 15065368; http://dx.doi.org/10.1038/nbt0904-767.

48. Ryffel GU. Orgenic plants: Gene-manipulated turf and forage grass (Paspalum notatum) for tolerance to herbicides. Pest Management Science 2010; 52:87-96; WOS:000283386700011; http://dx.doi.org/10.1007/s12095-009-0442-8.

49. Chen L, Liu YG. Male sterility and fertility restoration in crops. Annu Rev Plant Biol 2014; 65:579-606; PMID: 24313845; http://dx.doi.org/10.1146/annurev-plant-050213-041119.

50. Maliga P. Plantid transformation in higher plants. Annu Rev Plant Biol 2004; 55:289-313; PMID: 15137722; http://dx.doi.org/10.1146/annurev.arplant.55.031903.141633.

51. Moon HS, Abercrombie LL, Blanvillain R, Eda S, Formondo EP, Li Y, Nodoka M, Quilino RV, Ramanujam J, Ramanoharian F, Sajeev O, Sato T, Smith AG, Steiner EJ, Stewart CN Jr. Transgene excision in pollen using a codon-optimized serine residue dsp. Plant Reproduction 2013; 26:159-79; PMID: 23852378; http://dx.doi.org/10.1007/s11258-013-9340-2.

52. van Acker RC, Smulgański AR, Friesen LF. The potential benefits, risks and costs of genetic use restriction technologies. Can J Plant Pathol 2013; 35:2177-83; PMID: 23974935; http://dx.doi.org/10.1139/C13-059.

53. van Acker RC, Smulgański AR, Friesen LF. The potential benefits, risks and costs of genetic use restriction technologies. Can J Plant Pathol 2013; 35:2177-83; PMID: 23974935; http://dx.doi.org/10.1139/C13-059.

54. Saji H, Nakajima N, Aono M, Tamaoki M, Kubo A, Wakiyama S, Matsuo K. Monitoring the escape of transgenic oilseed rape around Japanese ports and road sides. Environmental bioassay research 2005; 4:217-22; PMID: 16827549; http://dx.doi.org/10.1016/j.jsbbs.62.124.

55. Nishizawa T, Nakajima N, Aono M, Tamaoki M, Kubo A, Saji H. Detection of ferl transgenic oilseed rape with multiple-herbicide resistance in Japan. Environ Biosafety Res 2006; 5:77-87; PMID: 17328854; http://dx.doi.org/10.1016/j.jsbbs.62.124.

56. Nishizawa T, Nakajima N, Aono M, Tamaoki M, Kubo A, Saji H. Monitoring the occurrence of genetically modified oilseed rape growing along a Japanese roadway: 3-year observations. Environ Biosafety Res 2009; 8:33-44; PMID: 19419652; http://dx.doi.org/10.1016/j.jsbbs.62.124.

57. Kawana M, Murakami K, Ishikawa T. Dispersal and persistence of genetically modified oilseed rape around Japanese harbors. Environ Sci Pollut Res Int 2009; 16:120-1; PMID: 1905951; http://dx.doi.org/10.1111/j.1199-1610.2009.00744.x.

58. Mitzuguti A, Yoshimura Y, Shibaike H, Matsuo K. Persistence of Feral Populations of Brassica napus originating from Spilled Seeds around the Koshima Seaport in Japan. Jap-Jap Agricultural Research Quarterly 2011; 45:181-5; http://dx.doi.org/10.6090/jarq.45.181.

59. Anno M, Wakiyama S, Naganuma M, Tamaoki N, Kubo A, Nishizawa T, Nakajima N, Tamaoki M, Kubo A, Saji H. Seeds of a possible natural hybrid between herbicide-resistant Brassica napus and Brassica rapa detected on a riverbank in Japan. gmcrops 2011; 2:201-10; PMID: 22179596; http://dx.doi.org/10.4161/gmcr.2.2.18931.

60. ritchie JS, Burt A. Hybridization in a commercial production field between imidazolinone-resistant winter wheat and joined grasses (Agropyron cylin drica) by imidazolinone-resistant wheat hybridization under field conditions. Weed Science 2008; 56:56-60; PMID: 19530257; http://dx.doi.org/10.1614/WS-D-09-00027.1.

61. Song X, Wang Z, Qiang S. Agromonic performance of F1, F2 and F3 hybrids between weedy rice and transgenic glufosinate-resistant rice. Pest Manag Sci 2011; 67:921-31; PMID: 21371956; http://dx.doi.org/10.1002/ps.2132.

62. Petty-Jones A, Martins BAR, Mallorey-Smith CA. Hybridization in a commercial production field between imidazolinone-resistant winter wheat and joined grasses (Agropyron cylin drica) by imidazolinone-resistant wheat hybridization under field conditions. Weed Science 2008; 56:56-60; PMID: 19530257; http://dx.doi.org/10.1614/WS-D-09-00027.1.

63. Knipl AL, McLachlan SM, van Acker RC, Friesen LF. Gene flow and multiple herbicide resistance in escaped canola populations. Weed Science 2006; 56:72-80; http://dx.doi.org/10.1614/WS-07-097.1.
88. Schaefer MG, Ross AA, Londo JP, Burdick CA, Lee EH, Travers SE, Van de Water PK, Sagers CL. The establishment of genetically engineered canola populations in the U.S. PLoS ONE 2011; 6:e25736; PMID: 21998689; http://dx.doi.org/10.1371/journal.pone.0025736

89. Yoshimura Y, Beckie HJ, Matsuo K. Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. Environ Biosafety Res 2006; 5:67-75; PMID: 17328853; http://dx.doi.org/10.1051/ebr:2006019

90. Warwick SI, Simard MJ, Legere A, Beckie HJ, Braun L, Zhu B, Mason P, Seguin-Swartz G, Stewart GN. Hybridization between transgenic Brassica napus L. and its wild relatives: Brassica rapa L., Raphanus raphanistrum L., Sinapis arvensis L., and Erucastrum gallicum (Wild.) OE Schulz. Theoretical and Applied Genetics 2003; 107:528-59; WOS:000185004200017; http://dx.doi.org/10.1007/s00122-003-1278-0

91. Simard MJ, Legere A, Warwick SI. Transgenic Brassica napus fields and Brassica rapa weeds in Quebec: sympatry and weed-crop in situ hybridization. Canadian Journal of Botany-Revue Canadienne de Botanique 2006; 84:1842-51; http://www.ingentaconnect.com/content/nrc/cjb/2006/00000084/00000012/art00010

92. Warwick SI, Legere A, Simard MJ, James T. Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy Brassica rapa population. Mol Ecol 2008; 17:1387-95; PMID: 17971090; http://dx.doi.org/10.1111/j.1365-294X.2007.03567.x

93. Quiti D, Chapela IH. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. Nature 2001; 414:541-3; PMID: 11734853; http://dx.doi.org/10.1038/35107068

94. Serratos-Hernandez JA, Gomez-Olivares JL, Salinas-Arreterna N, Buendia-Rodriguez E, Islas-Gutierrez F, De-Ira A. Transgenic proteins in maize in the soil Conservation area of Federal District, Mexico. Frontiers in Ecology and the Environment 2007; 5:247-52; http://dx.doi.org/10.1890/1540-9295(2007)5[247:TPIMIT|2.0.CO;2

95. Mercer KL, Wainwright JD. Gene flow from transgenic maize to landraces in Mexico: An analysis. Agriculture, Ecosystems & Environment 2008; 123:109-15; http://dx.doi.org/10.1016/j.agee.2007.05.007

96. Pinayo-Nelson A, Van Herwaarden J, Perales HR, Serranos-Hernández JA, Rangel A, Hufford MB, Gepts P, Garay-Arroyo A, Rivera-Bustamante R, Alvarez-Buylla ER. Transgenes in Mexican maize: molecular evidence and methodological considerations for GMO detection in landrace populations. Mol Ecol 2009; 18:750-61; PMID: 19143938; http://dx.doi.org/10.1111/j.1365-294X.2008.03993.x

97. Zapiola ML, Campbell CK, Butler MD, Mallory-Smith CA. Escape and establishment of transgenic glyphosate-resistant creeping bentgrass Agrostis stolonifera in Oregon, USA: a 4-year study. Journal of Applied Ecology 2008; 45:486-94; WOS:000241925000011; http://dx.doi.org/10.1111/j.1365-2664.2007.01430.x

98. Warnud LS, Lee EH, Fairbrother A, Burdick C, Reichman JR, Bollman M, Storm M, King G, Van de Water PK. Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. Proc Natl Acad Sci U S A 2004; 101:14533-8; PMID: 15448206; http://dx.doi.org/10.1073/pnas.0405154101

99. Ellstrand NC. Over a decade of crop transgenes out-of-place. In: Wozniak CA and McHughen, A., editors. Regulation of Agricultural Biotechnology: The United States and Canada. Springer Science+Business Media Dordrecht. 2012.

100. Bauer-Panskus A, Breckling B, Hamberger S, Then C. Cultivation-independent establishment of genetically engineered plants in natural populations: current evidence and implications for EU regulation. Environmental Sciences Europe 2013; 25:34; http://www.envurope.com/content/25/1/34