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# ExpertInnengutachten

Report for underpinning of the Austrian import bans for the oilseed rape lines Ms8xRf3 & GT73 under particular consideration of new scientific knowledge from international literature from 2011 to 2012



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# Summary of the recent scientific argumentation for the Austrian import bans of OSR

The prolongation of the Austrian import bans for the genetically modified herbicide tolerant (GMHT) oilseed rape (OSR) lines Ms8, Rf3 and Ms8xRf3 (BGBl. Nr. II 305/2010) and GT73 (BGBl. Nr. II 307/2010) are currently in force. New scientific evidence concerning the potential ecological impact of importing these genetically modified (GM) OSR lines is a prerequisite for the justification of continuing these import bans. Conclusions of this report are primarily based on recent scientific information, published in 2011 and 2012. However, the previous scientific justifications submitted by Austria to support the import bans of these GM OSR lines are still considered to be valid.

Several lines of scientific evidence identify **transport activities associated with the import of GM OSR** in countries with no GM OSR cultivation as a major factor for the unintended emergence and establishment of feral GM OSR populations outside cultivation areas. In this context, **accidental spillage of viable seeds during transport activities and seed handling** plays a significant role in enabling transgenes to enter the environment. Import activities of GM OSR might also be responsible for the introduction of GM OSR into the environment also in countries like Austria where no GM OSR is presently grown.

In the **EU**, no GM OSR is authorised for cultivation. Evidence for the widespread presence of feral non GM OSR populations is available from recent studies conducted in **France, Germany, the Netherlands, and the UK**. On the other hand in a study performed in **Switzerland** where neither cultivation nor import of GM OSR are presently allowed like in Austria, GM glyphosate tolerant OSR (GT73) was found in four of altogether 79 test sites, at the railway stations of Lugano (Canton Ticino), at the unloading railway yard in the port of Kleinhüningen (Canton Basel Stadt), at the railway station of Basel-St. Johann (Canton Basel Stadt) and at the railway yard in the port Muttenz-Auhafen (Canton Basel Landschaft). Its persistence has been attributed to contaminated seed spillage from freight trains since neither in Switzerland nor in Europe OSR GT73 is allowed to be grown. These findings confirm that seed spillage due to transportation of imported seed in countries without GM OSR cultivation is a main factor for the establishment of feral GM OSR along transport routes. In particular, railways are an ideal system where feral GMHT OSR plants are able to establish and spread from spilled seeds. The plants of these habitats are exposed to selective pressure in favour of herbicide resistance because herbicides are usually applied to keep the railways free from weeds. Hence, the establishment of these GMHT OSR populations is

encouraged. Due to the increasing presence of feral GM OSR on transport routes, intense weed management might become necessary in the future resulting in the repeated application of herbicides or the usage of more selective ones in order to be able to keep the railways free of feral GMHT OSR.

Earlier scientific findings of observed feral GMHT OSR populations in 2009 which have emerged due to seed spillage during transportation of imported GM OSR were confirmed in **Japan**, at that time a country without GM OSR cultivation but import of GM OSR. On 18 from 19 test-sites around Kashima seaport GM OSR populations identified in 2009 still showed continuous growth two years later. Similar to the situation in Switzerland, the feral GMHT OSR populations must have emerged from spilled GM OSR seeds during transportation. The examples from Switzerland and Japan are relevant for the Austrian justification because the emergence of feral GM OSR populations along transport routes is also likely for Austria if GM OSR seed would be imported.

Further evidence is contributed by countries where GM OSR is cultivated like in the **United States**. For the region of North Dakota, a first roadside survey was conducted which showed that feral OSR roadside populations – non GM as well as GM – are likely to persist, are capable of hybridising to produce novel genotypes and can contribute to the spread of transgenes outside cultivation areas. Feral OSR was detected at **45%** of the test sites along highways and expressways covering a sampling area of altogether 63.1 km. Of those, **more than 80%** of the feral OSR populations -- 41% positive for glyphosate resistance, 39% for glufosinate resistance, 0.7% containing both herbicide resistances -- were identified to be GM. About 87% among the tested plant individuals were sexually mature. OSR plant density ranged from 0 to 30 individuals/m<sup>2</sup>. In the examples from the United States where GM OSR is also cultivated seed spillage during transportation played a major role for the establishment of feral GM OSR populations. These observations underline the Austrian argumentation concerning the importance of accidental seed spillage during transport activities.

Furthermore, recent publications address the characteristics and the extent of unintended spillage of viable OSR grains into the environment during transport. A French study calculated an average seed spillage from grain trailers of **404±94 seeds per m<sup>2</sup>**. Studies from the United States and Japan show that a higher density of feral populations was registered at junctions between major roads, access points to crop fields and bridges and intersections of roadways with railway crossing. Moreover, roads with a paved surface supported seed spillage more than dirt roads. Hence, the main transportation routes for imported OSR seeds are more probable for adventitious seed spillage than unpaved field roads. Anthropogenic disturbance like mowing, herbicide application and soil disturbance as well as natural disturbance like flooding were shown to encourage the temporal persistence of feral OSR populations. Pet food production was identified as another possible way for accidental spillage of viable GM OSR grain.

**Gene flow** as another way of transgene dispersal has already been confirmed between GM OSR and several related species under natural conditions in recent publications. New scientific findings from Japan indicate that after seed spillage spontaneous hybridisation between GMHT *B. napus* and feral *B. rapa* has occurred. Studies conducted in the United States confirm that **HT traits accumulate and spread within feral OSR**. Feral OSR can function as stepping stones and form populations that accumulate transgenes. It was shown that transgenic traits are also able to increase plant fitness in habitats which are exposed to herbicide drift from glyphosate application on adjacent fields. Previous ecological risk assessment suggested that herbicide resistance traits have to be dealt with as neutral regarding the fitness of GMHT OSR in environments without herbicide application. However, new scientific results indicate that this assumption is not valid any more. Fitness costs/benefits resulting from single or stacked HT traits can be highly variable among different individuals. Hence, the significance of average fitness rates for risk assessment is limited and needs to be reconsidered.

Because of the required immense working and time effort, efficient management of feral GM OSR along transport routes will not be feasible especially in Austria where feral OSR is widespread over a variety of different habitats. Sites like road verges, field edges, railways as well as slightly inhabited open river banks will be the most preferred locations colonized by feral GM OSR. These habitats, although often underestimated in their ecological importance in agricultural landscapes, are endangered according to the Red List of Endangered Biotope Types in Austria and need to be conserved.

Small scale farming with fields less than 1 ha in size is typical for Austria. Extended consequences of spillage of GM OSR seeds have to be expected in small-scaled landscapes which are more vulnerable to cross-contamination due to scale issues. Moreover, in Austria a “zero-tolerance-policy” concerning seeds is laid down in an ordinance and feral GM OSR plants originating from GM OSR import therefore need to be regarded as an important factor which might contribute to the adventitious presence of GM OSR in conventional or GM-free OSR products. In addition, gene flow from these feral GM OSR plants to small non GM OSR fields should not be neglected in its impact as a contributor to GM contamination.

**Recent scientific findings mainly from 2011 and 2012 underline the Austrian justification against the import for the GMHT OSR lines Ms8xRf3 and GT73. Hence, the import bans of these lines are recommended to be prolonged in future.**

# Zusammenfassung der neuen wissenschaftlichen Argumentation für die österreichischen Importverbote für Raps

Die Verlängerungen der österreichischen Importverbote der gentechnisch veränderten herbizidresistenten (GVHR) Rapslinien Ms8, Rf3 und Ms8xRf3 (BGBl. Nr. II 305/2010) und GT73 (BGBl. Nr. II 307/2010) sind gegenwärtig in Kraft. Die Erbringung von neuen wissenschaftlichen Kenntnissen betreffend der potentiellen ökologischen Auswirkungen des Imports dieser gentechnisch veränderten (GV) Rapslinien ist eine Grundvoraussetzung für die Rechtfertigung, dass die österreichischen Importverbote aufrecht erhalten bleiben. Die Schlussfolgerungen dieses Gutachtens basieren hauptsächlich auf aktuellen wissenschaftlichen Kenntnissen, die zwischen 2011 und 2012 publiziert wurden. Aber auch die bisherigen wissenschaftlichen Rechtfertigungen, die von Österreich zur Untermauerung der Importverbote dieser GV Rapslinien vorgelegt wurden, werden weiterhin als gültig angesehen.

Verschiedene wissenschaftliche Beweislinien identifizieren **Transportaktivitäten, die mit dem Import von GV Raps** in Länder ohne GV Raps-Anbau verbunden sind, als einen Hauptfaktor für das unbeabsichtigte Auftreten und die Etablierung von verwilderten GV Rapspopulationen außerhalb des Anbaubereichs. In diesem Zusammenhang spielt die zufällige Verstreuerung von lebenden Samen während Transportaktivitäten und der Handhabung der Samen eine bedeutende Rolle. Auf diesem Weg ist es möglich, dass Transgene unbeabsichtigt in die Umwelt gelangen können. Der Import von GV Raps könnte auch in Ländern wie Österreich, in denen gegenwärtig kein GV Raps angebaut wird, das Eindringen von GV Raps in die Umwelt initiieren.

In der **EU** ist derzeit keine GV Rapslinie für den Anbau zugelassen. Wissenschaftliche Belege für das weitverbreitete Vorkommen von verwilderten nicht GV Rapspopulationen werden auch in aktuellen Studien, die in **Frankreich, Deutschland, den Niederlanden und Großbritannien** durchgeführt wurden, erbracht. Andererseits wurde in einer Studie in der **Schweiz**, ein Land wie Österreich, in dem weder der Anbau noch der Import von GV Raps gegenwärtig erlaubt ist, GV Glyphosat resistenter Raps (GT73) an vier von insgesamt 79 Teststellen identifiziert. Die Fundorte waren der Bahnhofsbereich von Lugano (Kanton Tessin), der Hafen von Kleinhüningen (Kanton Basel Stadt), der Bahnhof von Basel-St. Johann (Kanton Basel Stadt) sowie der Auhafen Muttenz (Kanton

Basel Landschaft). Das Auftreten dieser GV Rapspflanzen wurde auf die Verstreung von kontaminierten Rapssamen von Güterzügen zurückgeführt, da die Rapslinie GT73 weder in der Schweiz noch in der Europäischen Union angebaut werden darf. Diese Funde bestätigen, dass Samenverstreung im Zuge des Transports von importierten Samen in Länder, in denen GV Raps nicht angebaut wird, einen Hauptfaktor für die Etablierung von verwilderten GV Rapspopulationen entlang von Transportrouten darstellt. Gerade Bahngleiskörper sind ideale Systeme, in denen sich verwilderte GVHR Pflanzen, die aus verstreuten Samen hervorgegangen sind, etablieren und ausbreiten können. Die Pflanzen dieser Lebensräume profitieren von ihrer Resistenz gegenüber den entlang der Bahntrassen ausgebrachten Herbiziden. Demzufolge wird die Etablierung dieser GMHR Rapspopulationen begünstigt. Aufgrund des anwachsenden Aufkommens von verwildertem GV Raps entlang von Transportrouten wird in Zukunft ein noch intensiveres Unkrautmanagement erforderlich werden, das in einer wiederholten Herbizidapplikation oder der Anwendung von selektiveren Herbiziden resultieren könnte, um Bahngleiskörper auch weiterhin frei von GVHR Raps halten zu können.

In **Japan** wurden 2009 GVHR Rapspopulationen, die auf Samenverluste während des Transports von importierten GV Raps zurückgeführt wurden, festgestellt. Zu diesem Zeitpunkt wurde in diesem Land kein GV Raps angebaut sondern lediglich importiert. An 18 von insgesamt 19 Teststellen um den Hafen Kashima zeigten GV Rapspopulationen, die in den Untersuchungen von 2009 identifiziert wurden, auch zwei Jahre später noch beständiges Wachstum. Ähnlich der Situation in der Schweiz wird angenommen, dass die verwilderten GVHR Rapspopulationen von Transport verstreuten Samen hervorgegangen sind. Die Untersuchungsergebnisse aus der Schweiz und aus Japan besitzen auch für die österreichische Rechtfertigung Relevanz, da das Auftreten verwilderter GV Rapspopulationen entlang von Transportrouten im Falle eines Imports von GV Rapssamen auch für Österreich zu erwarten wäre.

Weitere wissenschaftliche Nachweise werden von Ländern geliefert, in denen GV Raps angebaut wird, wie es etwa in den **USA** der Fall ist. Eine Erhebung der Straßenränder wurde erstmalig in der Region von North Dakota durchgeführt. Im Zuge dieser Überprüfung wurde festgestellt, dass verwilderte Rapspopulationen (nicht GV und GV) entlang von Straßenrändern mit großer Wahrscheinlichkeit vorkommen. Im Falle einer Hybridisierung mit verwandten Arten und der Produktion neuer Genotypen tragen sie zur Verbreitung von Transgenen in die Umwelt bei. Verwilderter Raps wurde auf **45%** der Testpunkte entlang einer Gesamtuntersuchungsstrecke von 63,1 km an Schnellstraßen und Autobahnen festgestellt. Von diesen Rapspflanzen wurden **mehr als 80%** als GV identifiziert. 41% waren Glyphosat-resistent, 39% Glufosinat-resistent und 0,7% wiesen beide Herbizidresistenzen auf. Etwa 87% von den getesteten Pflanzenindividuen waren in fortpflanzungsfähigem Stadium. Die Populationsdichte erreichte Werte bis 30 Individuen/m<sup>2</sup>. In den wissenschaftlichen Untersuchungen der USA, ein Staat mit GV Raps-Anbau, spielte Samenverstreung während des Transports eine

wichtige Rolle für die Initialisierung und Etablierung von verwilderten GV Rapspopulationen. Diese Beobachtungen unterstreichen die österreichische Argumentation, mit der immer wieder auf die Bedeutung von zufälliger Samenverstreung während des Transports hingewiesen wird.

Darüber hinaus wird in den aktuellsten Publikationen das Ausmaß von unbeabsichtigten Verstreungen von lebenden Rapsamen während des Transports in die Umwelt behandelt. In einer französischen Studie wurde beim Samentransport in Lastwagenanhängern ein durchschnittlicher Verlust von **404±94 Samen pro m<sup>2</sup>** berechnet. Studien, die in den USA und Japan durchgeführt wurden, zeigen, dass größere Populationsdichten von Ruderalraps an Kreuzungen von Hauptstraßen, Zugangspunkten zu Feldern und Brücken sowie Kreuzungen von Straßendämmen und Bahngleiskörpern beobachtet werden konnten. Im Unterschied zu unbefestigten Transportwegen begünstigen Straßen mit ihrer Oberfläche die Samenverstreung. Infolgedessen ist die zufällige Verstreung importierter Samen auf Haupttransportrouten eher zu erwarten als auf unbefestigten Feldwegen. Anthropogene Störungen (z. B. Mahd, Herbizidanwendung, Bodenbearbeitung) wie auch natürliche Ereignisse (z. B. Überflutung) können die Persistenz von verwilderten Rapspopulationen fördern. Die Produktion von Haustierfutter wurde als ein weiterer möglicher Faktor der zufälligen Verstreung von lebenden GV Rapsamen identifiziert.

**Genfluss** zwischen GV Raps und nah verwandten Arten unter natürlichen Bedingungen wurde als zusätzliche Ausbreitungsquelle von Transgenen in den Publikationen von 2011 und 2012 erneut bestätigt. Neue wissenschaftliche Funde aus Japan deuten darauf hin, dass nach der Samenverstreung spontane Hybridisierung zwischen GVHR *B. napus* und verwildertem *B. rapa* aufgetreten ist. Studien, die in den USA durchgeführt wurden, bestätigen, dass **HR Eigenschaften akkumulieren und sich innerhalb von verwildertem Raps** ausbreiten können. Verwilderter Raps kann als Trittstein fungieren und Populationen bilden, die Transgene akkumulieren. Es wurde zudem gezeigt, dass transgene Eigenschaften dazu beitragen, die Fitness von Pflanzen in jenen Habitaten zu erhöhen, die der Herbizid-Verdriftung von Glyphosat aus benachbarten Feldern ausgesetzt sind.

In älteren ökologischen Risikoabschätzungen wurde vorgeschlagen, Herbizidresistenz-Eigenschaften bezüglich der Fitness von GVHR Raps in Lebensräumen ohne Herbizidanwendung neutral zu bewerten. Neue wissenschaftliche Ergebnisse deuten allerdings darauf hin, dass diese Annahme nicht mehr gültig ist. Fitness-Nachteile/Vorteile einzelner oder auch mehrerer („stacked genes“) HR Eigenschaften können zwischen verschiedenen Individuen sehr variabel sein. Folglich ist die Aussagekraft von durchschnittlichen Fitnessraten für die Risikoabschätzung limitiert und muss neu überdacht werden.

Ein effektives Management von verwildertem Raps entlang von Transportrouten wäre besonders in Österreich, wo dieser weitverbreitet in diversen Habitaten

auftritt, aufgrund des erforderlichen immensen Arbeitsaufwandes und der damit verbundenen Kosten nicht realisierbar. Lebensräume wie Straßen- und Feldränder, Gleiskörper und auch lückig bewachsene Flussufer sind Standorte, die von verwildertem GV Raps bevorzugt besiedelt werden. Diese Biotope in den Agrarlandschaften werden oftmals in ihrer ökologischen Bedeutung unterschätzt. In der Roten Liste der gefährdeten Biotoptypen Österreichs sind einige dieser mit unterschiedlichem Gefährdungsgrad angeführt und müssen erhalten werden.

Für Österreich ist eine kleinstrukturierte Landwirtschaft mit Feldgrößen unter 1 ha typisch. Weitreichende Folgen der Verstreuung von GV Samen werden vor allem in kleinteiligen Landschaften erwartet, die aufgrund größenabhängiger Faktoren anfälliger für Kreuz-Kontamination sind. Zusätzlich gilt in Österreich eine „Null-Toleranz-Politik“ bei Saatgut, welche in Form einer Verordnung in Kraft ist und sich deshalb verwilderte GV-Rapspflanzen aus Importverlusten von transgenem Raps für die Kontamination von konventionellen bzw. gentechnikfrei erzeugten Produkten verantwortlich zeichnen können. Darüber hinaus darf der mögliche Genfluss von verwilderten GV Rapspflanzen auf kleine nicht GV Rapsfelder als unbeabsichtigte GV Kontamination nicht vernachlässigt werden.

**Die österreichischen Importverbote der Rapslinien Ms8xRf3 und GT73 sind nach wie vor wissenschaftlich gerechtfertigt und werden darüber hinaus durch aktuelle Studienergebnisse von 2011 und 2012 untermauert. Es wird deshalb dringend empfohlen, die Importverbote auch in Zukunft weiterhin beizubehalten.**

# Introduction

In Austria, the demand of oilseed rape (OSR) is not covered by domestic cultivation. Consequently, OSR has to be imported to Austria. In 2011, altogether 233,161.35 t OSR or turnip seeds were imported from 15 countries which included ten EU member states, Croatia, Serbia, Switzerland, Chile, and Singapore (Außenhandel der Statistik Austria). The main exporting countries were Hungary (128,731.78 t), Slovakia (52,229.13 t), and the Czech Republic (32,404.65 t). Import of OSR and turnip seeds increased from 99,114 t in 2004; 113,119 t (1.-3. quarter of 2005); 200,146 t (2006); 238,683 t (2007) up to 268,908 t in 2008 (update 2008 of Reiner 2006). In 2008, small amounts of seeds were imported from Canada, China, Russia, and the Ukraine. In 2011, however, none of the four mentioned countries exported OSR or turnip seeds to Austria any more. The only country of export in 2011 cultivating GM canola was Chile (JAMES 2011). The current data from the Statistik Austria show a similar situation of import activity compared with the previous years. Peripherally located oil mills will still become more important in future (REINER 2006), a situation which will extend the way of transportation of imported OSR seeds and hence, become more difficult to be analysed. Moreover, OSR usage could increase in near future in the European Union, e. g. due to the usage as biofuel (EUROPEAN COMMISSION 2006). Hence, also import activities of OSR to Austria are expected to increase further on in future, although the total amount of imported seeds decreased in 2011.

The prolongation of the Austrian import bans for the genetically modified herbicide tolerant (GMHT) oilseed rape (OSR) lines Ms8, Rf3 and Ms8xRf3 (BGBl. Nr. II 305/2010) and GT73 (BGBl. Nr. II 307/2010) are currently in force. Concerns regarding the environmental safety of the import of these genetically modified (GM) lines still exist. Widespread feral GM OSR populations arising from seed spillage during transportation -- especially along transport routes like roads, railways, rivers as well as at points of landing, storage facilities and crushing plants --, their persistence and their high potential for cross-fertilisation pose a potential risk for the adventitious spread of transgenes into the environment. Recent scientific publications concerning the relevance of transport activities of imported GM OSR seeds as source for the establishment of feral GM OSR populations underline these concerns.

*Brassica napus* L. is a comparable young crop which has been domesticated during the last 300-400 years. Hence, its traits of a wild plant such as seed shattering and partial seed dormancy are still expressed in commercial OSR. These traits of its wild parents, *B. oleracea* (cabbage) and *B. rapa* (turnip), make it a competitive crop also outside its cultivation area. Consequently, OSR has a large potential to establish and persist as a feral plant. Transgenes of the feral plants might also enter semi-natural or natural habitats. Hybridisation of feral GM OSR with sexually compatible

species is an additional way for the escape of transgenes into the environment. The transgenes will then undergo evolutionary dynamics. In several, previously published international studies seed spillage during transport activities was identified as a cause for the establishment of feral OSR populations **on roadside verges, in railway habitats, at construction sites**, and particularly in areas where seed intended for planting as well as commodities that can function biologically as seed is handled like **port areas, storage facilities and crushing plants**. Seed dispersal in general is a complex phenomenon. Anthropogenic activities enhance the natural dispersal capacity of plant seeds significantly in quantity as well as in distance (BAILLEUL et al. 2012). Specifically agricultural machines and transport trucks are able to disperse significant quantities of viable OSR grain into the environment. In case of OSR, seed spillage from grain trailers as well as from trains during transportation is of major importance.

Hybridisation and polyploidisation are key processes which drive the evolution of the entire Brassicaceae family. In the context of GMOs, OSR is regarded as a crop with a high potential to cause environmental and economical problems because volunteers and feral plants containing GM traits will persist outside cultivation areas (REUTER & al. 2011). GM OSR might influence population ecology of wild species by introducing novel traits which may enhance the fitness under specific conditions. Therefore, they could lead to detrimental effects such as the extinction of native alleles or the decline of natural populations. In the following report, recent data reported in scientific papers particularly in 2011 and 2012 are discussed. This new evidence supports concerns which motivated the introduction of the Austrian safety measures regarding the GM OSR lines GT73 and Ms8, Rf3 and Ms8xRf3 (Notification C/BE/96/01).

# Distribution pathways of transgenes

## Accidental spillage during transport activities

### Previous Austrian justification for the GM OSR import ban

Accidental OSR seed spillage during transport activities of the import of the GMHT OSR lines GT73 and Ms8xRf3 into the EU from non-EU production areas were identified as a major ecological risk for GM contamination in the previous scientific justification of the Austrian safety measures. This concern is supported by several recent scientific publications which have appeared particularly between 2011 and 2012 and are discussed in the following section.

### Persistence and frequency of oilseed rape populations outside fields

#### Previous scientific findings

Origin, local occurrence, persistence, and population dynamics of feral OSR populations have already been studied in several European countries (e.g. PASCHER et al. 2000, 2006, 2010, 2011; PESSEL et al. 2001; CRAWLEY & BROWN 2004; MENZEL 2006; DIETZ-PFEILSTETTER et al. 2006; PIVARD et al. 2007, 2008; GARNIER et al. 2008, OWEN 2008; REUTER et al. 2008; ELLING et al. 2009; MIDDELHOFF et al. 2009; BANKS et al. 2010; SQUIRE et al. 2010). Moreover, several papers deal with this issue in other regions than Europe (e.g. Canada: YOSHIMURA et al. 2006; KNISPEL et al. 2008; BECKIE & WARWICK 2010; KNISPEL & MCLACHLAN 2010; Japan: KAWATA et al. 2009; NISHIZAWA et al. 2009; United States: SAGERS et al. 2010). In the following sections relevant findings from research during the last two years are summarised.

#### Recent studies of feral OSR arising from seed spillage during transportation (scientific papers between 2011 and 2012)

Feral OSR has become widespread in **Europe** on waysides and waste ground (SQUIRE et al. 2011). The data of feral abundance and crop yield of OSR from five established demographic studies in agricultural habitats in **Denmark, Germany (2), France**, and in the **UK** were combined. The aim of this meta-study was the investigation of persistence of **feral non GM OSR populations** over time in different European areas. Feral plants were found in all five test regions forming populations of 0.2 to 15 per km<sup>2</sup>. The seeds from such feral populations amounted to <0.0001% of the seeds produced by cultivated OSR in each region. The size of the tested OSR populations varied between single plants up to more than 1,000 individuals with the majority of populations consisting of around 100 plants. Feral OSR showed a widespread capacity to persist in all five test regions. In comparison with the contribution by cross pollination between fields and by volunteer weeds in fields, feral OSR was no relevant source of macroscopic impurity in crops at its present density in these countries. However, feral OSR opens up the opportunity for genetic recombination, stacking of genes, and the evolution of genotypes that

could lead to increased costs of weed control in the future. Feral GM OSR populations might pose a long term risk of contaminating conventional crops under strong selective pressure like herbicide application.

**Switzerland** has implemented a moratorium on the cultivation of GMOs until the end of November 2013. A monitoring system is proactively established in order to identify GM plants in the environment. For this purpose several studies have been commissioned. The focus of the monitoring is put on transportation routes. In the recently performed studies railway stations and areas of Switzerland and the Principality of Liechtenstein were investigated for the **accidental presence of GM glyphosate tolerant (GT) OSR** (SCHOENENBERGER & D'ANDREA 2012). Switzerland does neither import nor cultivate transgenic OSR (SWISS FEDERAL OFFICE FOR AGRICULTURE 2011; SWISS FEDERAL OFFICE OF PUBLIC HEALTH 2011). Sampling was part of the monitoring program of GM organisms under the authority of the Bundesamt für Umwelt (BAFU). SBB Railways were monitored from the Italian and French border, respectively, to the oilseed processing plants in the Cantons of Ticino and Basel-landscape in spring 2012. Altogether, 79 railway stations and areas were investigated for the presence of feral OSR populations. In 58 of these locations (**73%**), feral OSR was present. 50 of the altogether 2403 plant individuals tested for glyphosate resistance using immunologic test kits that are **2.1%, proved to be transgenic**. The "Kantonales Labor des Gesundheitsdepartments Basel-Stadt" (KL BS) confirmed the presence of GMHT OSR of the variety GT73 using the event specific PCR test. The GM plants were sampled at four locations. At the railway station of Lugano (Canton Ticino) 91.3% of the tested OSR plants (21 from 23 tested individuals) expressed the CP4 EPSPS protein. Moreover, at the unloading railway yard in the port of Kleinhüningen (Canton Basel Stadt) 88.9% (16 from 18) of the plants, at the railway station of Basel-St. Johann (Canton Basel Stadt) 92.3% (12 from 13), and at the railway yard in the port Muttenz-Auhafen (Canton Basel Landschaft) 2.1% (1 from 47) tested positive. In two of these sites -- Lugano and Kleinhüningen -- GM OSR plants survived glyphosate application. The authors suggest that the plants at these two locations "were expressing the transgenes at efficient levels" (SCHOENENBERGER & D'ANDREA 2012; page 7). All Swiss railroad companies use glyphosate for weed control. The populations were probably introduced through spillage of contaminated seed from freight trains or during the transfer of goods from cargo ships to trains, since neither in Switzerland nor in Europe such GM OSR varieties are allowed to be grown. The authors assume that the feral OSR populations have probably established through introduction of only a few or even one single seed containing the transgene, followed by multiple cycles of reproduction. Although transgenic feral OSR has been found on four of altogether 79 tested railway areas it is conceivable that it will be just a matter of time for these transgenic populations to become more abundant especially under the positive selective pressure when herbicide is applied. Bird feeding on the lost seeds was observed in the railway areas during the survey. Birds are a main vector for long distance transport of seeds and hence could support the dispersal of these transgenic populations even outside the railway areas.

In 2009, **Japan** started to cultivate GMHT *B. napus* only to a very small extent. GM OSR is imported into this country mainly from Canada (2009: 94.4%; NISHIZAWA et al. 2010), between 2004 and 2008 annually around two million tons. Studies were carried out to confirm the establishment and persistence of feral GM OSR. During the last years feral GMHT OSR was found growing around some major Japanese seaports. Their presence around the Kashima seaport was assumed to trace back to import of seeds for food and feed from foreign countries (SAJI et al. 2005; NISHIZAWA et al. 2009, 2010). MIZUGUTI et al. (2011) investigated the persistence of the feral OSR populations counting the number of growing plants each month within a time period from July 2004 to December 2005, a time period in which no GM OSR was grown in Japan, and the “self-sustainment of the populations” at 19 sites around Kashima seaport. The continuous growth of feral *B. napus* plants was recorded **at all sites except one**. The number of plants per population varied among sites and survey months and ranged from **0 to 202**. Competition with other species and frost-kill were identified to be responsible for the reduction in plant number at two sites. But the main factors for plant disappearance were mowing and herbicide application. The origin of the feral plants was probably seed spillage from trucks or from a seedbank, an issue which is consistent with the results of previous studies. In a study of AONO et al. (2011) plant samples were collected from twelve areas near major ports through which GM OSR is imported into Japan. The presence of glyphosate and/or glufosinate-resistant *B. napus* was confirmed in eight of the twelve tested areas. The area around Yokkaichi was affected mostly. Several GMHT plants were detected not only on the roadside where OSR was spilled during transport processes but also on riverbanks where also feral populations of *B. rapa* and *B. juncea* grew. OSR plants which were tolerant to both herbicides were identified in four continuous years from 2005 to 2008 in that area. 2008, seeds of a possible natural hybrid between GMHT OSR and *B. rapa* sampled at a Yokkaichi site were tested for herbicide resistance. Some seedlings derived from these seeds showed glyphosate resistance. These observations strongly suggest that **hybridisation between HR OSR and feral *B. rapa* has occurred spontaneously**. Hybrids between transgenic *B. napus* and *B. juncea* were not detected. The presence of GMHT *B. napus* and its hybrids has been confirmed only along a major transportation road as well as on riverbanks directly beneath bridges of the traffic system.

Another example for a feral OSR population growing on a riverbank was detected along the river Kamp in **Austria** in August 2011 (K. PASCHER, personal observation, figure 1). OSR seeds can be transported on the water surface along rivers over very long distances. They are able to establish also in large populations on nearly colonized sites. As discussed before, riverbeds were also confirmed by the Japanese authors to be particularly important for the occurrence of feral GM OSR plants. In Japan, some roads used for land transportation of imported OSR seeds cross large rivers. Due to seed spillage during transportation of GM OSR on these crossings, feral GM OSR populations would be expected to establish on riverbanks too. Also the Austrian traffic network used for transportation has such crossings

which would enable establishment of feral OSR on riverbanks. In case of import of GMHT OSR, spillage during transport on these sites would enable transgenes to enter semi-natural habitats like riverbanks.



**Figure 1:** Large feral OSR population on a riverbank along the river Kamp in Lower Austria (observation and photo: K. PASCHER, August 2011).

Demographic studies across different European countries indicate that feral OSR populations are frequently present and are able to act as a genetic bridge between old and present OSR varieties (PASCHER et al. 2010; DEVOS et al. 2012).

### **Recent studies from feral OSR arising from seed transport during OSR cultivation activities (papers between 2011 and 2012 considered)**

The following studies which focus on OSR cultivation are discussed within the context of OSR import because the experience from transport events in general can also be applied to the OSR import issues.

In a **French study** (BAILLEUL et al. 2012) the amount of seed spillage from grain trailers during harvest has been quantified using 85 installed seed trap-sites on the road verges in an agricultural area around the grain silo of Selommès (Loir-et-Cher). The amount of spilled seeds decreased with the distance from the trap-side to the road verge and to the nearest field. Moreover, fewer seeds were found with increasing distance to the grain silo (compare PASCHER & DOLEZEL 2005). No effects of the number of road lanes were visible as well as of the road type. Cumulative effects were identified for one-lane roads. It was shown that grain trailers frequently dispersed seeds on road verges up to a distance of 400 m from an OSR field. An average seed spillage of **404±94 seeds per m<sup>2</sup>** was calculated from the collected data (a total of 7,710 seeds trapped within eight days). On a 5 km road that serviced 66 ha of OSR fields the seed spillage on the road verges in the transportation direction was estimated to be nearly two million OSR seeds. The largest amounts of spilled seeds could be identified on a road where no feral OSR population was observed during monitoring in the previous ten years (20% of the total number of collected seeds). Currently prevailing conditions of the road verge habitat that are able to favour or prevent the germination of spilled seeds and the subsequent emergence of feral OSR plants could be the reason for these observation (COLBACH 2009).

In the **United States** more than 90% of OSR fields are cultivated with GMHT OSR. Feral OSR populations are present in the USA, they are large and widespread (SAGERS 2012). Moreover, flowering times as well as the high fertility of the tested feral OSR populations suggest that these populations are established and persist outside of cultivation (SCHAFER et al. 2011). A roadside survey on the extent of occurrence of feral OSR populations in **North Dakota**, the dominant growing region of the United States, was conducted. Random samples of OSR plants from verges along highways and expressways over a distance of around 5,600 km were taken. The sampling area covered 63.1 km of roadside habitats of altogether 5,600 km road way. Leaf fragments were tested for the presence of the proteins conferring tolerance to glyphosate (Roundup) or glufosinate (Liberty). Feral *B. napus* was present at **45% (288/634)** of the road survey sampling sites. Of those, **80% (231/288)** expressed at least one of the transgenes. **41% (117/288)** proved to be positive for **glyphosate resistance**, **39% (112/288)** for **glufosinate resistance** and **0.7% (2/288)** expressed both forms of herbicide resistance. Densities at sampling sites ranged from **0 to 30 plants/m<sup>2</sup>**. Among the achieved specimens, **86.8% were sexually mature** (flower buds to mature fruits with seeds). The big difference in flowering phenology suggested that flowering canola in roadside habitats may have originated from the previous generation's seedbank rather than from seed spillage during the current growing season. Furthermore, populations of GM OSR were denser along major transport routes, especially at construction sites. At a finer scale, feral populations grew denser at junctions between major roadways, access points to crop fields and bridges, and intersections of roadways with railway crossing. At these sites, seed spillage during transport is a likely mechanism for dispersal. This observation is in accordance with the findings in Japan. Feral populations were occasionally found at remote locations far from OSR production, transportation, or processing facilities. Also **mowed or herbicide treated roadsides** were colonized. In all randomly tested large populations a mix of both herbicide resistant phenotypes was identified among different plants which probably trace back to multiple seed spillage events. The feral OSR populations are also able to colonize habitats which plant communities are exposed to selection pressure.

Feral GM OSR in North Dakota was recently also monitored in **California** by MUNIER et al. (2012). GM glyphosate-resistant OSR populations were found on combined loading areas as well as along country roads where the combine was hauled. Unexpected OSR roadside populations were found even during the winter in 2009. California's diverse agriculture and restrictions on phenoxy herbicide use makes weedy glyphosate resistant OSR control much more difficult than in other countries like Canada or the northern cereal growing areas of the United States for example. Mowing or herbicide use performed too early in the season proved to encourage establishment of the OSR populations due to the removal of competing vegetation (KNISPEL & MCLACHLAN 2010). As earlier shown by CRAWLEY & BROWN (2004) also soil disturbance might encourage the frequency of OSR populations under certain circumstances. Regularly disturbed habitats -- anthropogenic (mowing, herbicide application, soil disturbance) as well as natural disturbance

(flooding) -- can encourage persistence of feral populations for longer periods (GARNIER et al. 2006, DEVOS et al. 2012).

Also Monsanto attributed the high number of feral GM OSR plants along road verges to spillage events along transport routes ([www.biosicherheit.de](http://www.biosicherheit.de)).

For the estimation of seed spillage during transport the mode of product transfer, the intended use, the volume of imported seed, the share of GMHT OSR in imported commodities as well as the country of origin are essential to be evaluated (DEVOS et al. 2012). At present, Austria imports OSR seeds from 15 different countries, including only one country, Chile, where GM OSR is grown. TAMIS & DE JONG (2010) investigated the transport chain in the **Netherlands** in order to better estimate the likelihood of feral populations arising from seed spillage during transport activities. The following key points for seed losses were identified by the authors: seed spillage occurs during quayside unloading, overland truck transport to storage facilities, especially for the small crushing plants, and disposal of seed cleaning waste. In this respect, the cleaning procedure of seeds which are then used for the production of pet food, also including seed mixtures for birds, is a probable way for GM seed loss. Movement of vehicles has repeatedly been confirmed as a main source of OSR seed transport and the seed infestation of new sites along roadways by MUNIER et al. (2012). In this context, paved road surfaces and areas close to grain elevators proved to be more likely to contain populations of GMHT OSR than dirt roads or areas further away from grain elevators (KNISPEL & MCLACHLAN 2010).

## Gene flow

### Previous Austrian justification for the GM OSR import ban and previous scientific findings

Hybridisation is another way for transgenes to enter the environment. The aspects summarized in the following are the prerequisites for a successful hybridisation of two sexually compatible species: Flowering of both hybridisation partners must overlap at least partly in time and space. Hybridisation partners have to share common pollinators to exchange pollen or are wind-pollinated. There should be only low genetic barriers between the two genomes. Within the family Brassicaceae interspecific hybridisation is a common feature. Approximately twenty species have been identified to show at least some degree of sexual compatibility with *B. napus* (PASCHER & GOLLMANN 1999; CHEVRÉ et al. 2004; SAGERS 2012). Gene flow between *Brassica napus* and closely related species as well as the introgression of the transgenes of feral GM OSR to certain wild relatives have already been confirmed in several publications (e.g. CHEVRÉ et al. 2004; YOSHIMURA et al. 2006; HÜSKEN & DIETZ-PFEILSTETTER 2007; WARWICK et al. 2008; DEVOS et al. 2009; DI et al. 2009; DIETZ-PFEILSTETTER & ZWERGER 2009; ELLING et al. 2010) and can therefore not be excluded under Austrian conditions. Recent findings from 2011 to 2012 are presented in the following.

## Recent scientific findings (2011-2012)

There is recent evidence that **insects** play a role in long-distance OSR-pollination and may induce gene escape from GM fields, even when the insect population density in a field is relatively low (CRESSWELL 2008). In a study of CHIFFLET et al. (2011) a large variety of insect species including bees, syrphid flies, sawflies, and sphecid wasps was identified to transfer viable pollen among OSR plants over considerable distances up to **1.1 km**. 39.4% of the tested insects on male-sterile flowers carried OSR pollen. Insect size was the only significant factor pertaining to the insects that explained the seed-set success.

***Brassica rapa*** (turnip, birdseed rape), one parent of OSR, is weedy throughout North-America (SAGERS 2012). *B. rapa* populations may be marginalized by competing populations of canola and canola x weed hybrids because they were shown to produce more seeds than turnip. The hybrids proved to be big vital plants.

VACHER et al. (2011) assessed the impact of interspecific hybridisation between transgenic ***B. napus*** and weedy ***B. rapa*** on the evolution of the weedy phenotype. Weedy individuals that flowered later and for longer periods were more likely to receive transgenic pollen from crops and weed x crop hybrids. Because stem diameter is correlated with flowering time, plants with wider stems were also more likely to be pollinated by transgenic plants. The analyses of VACHER et al. (2011) show that differences in phenology between weedy *B. rapa* and GM OSR are likely to alter the phenotypic structure of weed populations by promoting interspecific hybridisation in only a subset of weedy plants with specific phenotypes and by altering the fitness of hybridising weeds.

## Relevance for supporting the Austrian import-bans – distribution pathways of transgenes

Adventitious GM seed spillage during transportation, contamination of non GM OSR seed due to gene flow of feral GM OSR populations and gene flow to sexually compatible species of *B. napus* are possible ways for transgenes to enter the environment.

**Seed spillage during transport activities** is a major issue for the initialisation process of feral OSR populations. This argumentation of the previous Austrian justification for the GM OSR import bans is supported by several recent publications. Feral GMHT OSR populations emerging from seed spillage events during import activities could repeatedly been found in port areas in Japan over years (MIZUGUTI et al. 2011). The issue that regional occurrence, frequency and population sizes of feral OSR populations differ in different regions was demonstrated in a recent publication from SQUIRE et al. (2011) which identified feral OSR population size differences between single plants up to more than 1,000 individuals in five different regions in Europe (Denmark, France, Germany (2), UK).

The authors calculated that the produced seeds of feral OSR accounted for <0.0001% of the cultivated OSR seed in each test region, **one feral plant seed for 1,000 000 crop seeds only**. At a first sight an admixture share of <0.0001% sounds low but if the exact number of seeds produced by feral OSR is calculated for Austria this would mean **58.8 millions (58 800 000) of feral OSR seeds** as annual admixture input for Austria with a number of commercially produced seeds in 1999 from the total OSR cultivation area of approximately 58.8 trillions (58 800 000 000 000, PASCHER 2001). DEVOS et al. (2012) state that the contribution to gene flow from feral OSR plants should be negligible compared to that from crop plants and volunteers. The only exceptions to this might be where occasionally very large populations of feral plants (e.g., >10,000 plants) occur in derelict fields or around major construction works, adjacent to very small oilseed rape crop fields or oilseed rape certified seed production fields, or in regions where a 'zero-tolerance' policy in terms of GM admixtures is in place (DEVOS et al. 2012: page 11). These exceptions totally apply to the Austrian situation with its small fields and the high standards in OSR production with a predefined "zero-tolerance" of GM contamination in seeds (BGBl. II Nr. 478/2001).

The establishment and persistence of GMHT OSR (canola) populations outside cultivation were proven for the United States (North Dakota) for the first time, more than a decade after its commercial release which raises uncertainties on the applicability and effectiveness of existing monitoring programs in the States (SCHAFER et al. 2011).

It was confirmed in several recent publications that road verges, railways and also riverbanks of running waters are preferentially colonized by feral OSR. Main roads with a paved road surface were identified to support spillage events during transport activities in contrast to dirt roads. No effects of the number of road lanes were visible. Furthermore, embark points, storage ground, crushing plants as well as areas close to grain elevators also proved to be particularly appropriate for the establishment of feral OSR plants. It was determined that feral populations grew denser at junctions between major roadways, access points to crop fields and bridges, and intersections of roadways with railway crossing. Running waters are effective ways for seed transport and could also pose a suitable way of transport route in the context of GM seed import. Also mowed or herbicide treated roadsides were colonized although under anthropogenic control.

**Railways** are a highly interlinked habitat, where accidental spillage of transported OSR seeds from trains occurs regularly. In the Swiss study of SCHOENENBERGER & D'ANDREA (2012) feral GMHT OSR could be identified at four railway areas which originated from contaminated non GM OSR seed. The herbicide glyphosate is regularly applied to control the weeds along railways in Switzerland which is similar to Austria. Here the herbicide is regularly sprayed on railways by the Austrian Railway Company at least once a year with different intensities (SATTELBERGER 2001). This circumstance increases the possibility for establishment of plants which are

resistant to this herbicide. In case of the establishment of GMHT OSR plants it might become necessary to spray herbicides three to four times to keep the railways free of weeds. This enhanced measure could increase selection pressure as it is already known from GM OSR cultivation.

Ruderal sites will be the main affected areas of feral GM OSR. These sites play an essential role as a refuge for various animal and plant species especially within intensively used agricultural areas. In Austria, they are included in the Red List of Endangered Biotope Types assigned to different degrees of threat.

OSR is assumed to be one of the most worrisome crops regarding **gene flow**. It was already proven for several times that gene flow regularly occurs between OSR and several related species which are sexually compatible. A recent study demonstrates that cross-fertilisation due to pollinators occur at low frequencies even over several kilometres (CHIFFLET et al. 2011). The introgression of a HT (glyphosate) transgene from OSR into its weedy relative *Brassica rapa* discussed in the previous Austrian justification is confirmed by a recent study from AONO et al. (2011). The authors detected seeds of a possible natural hybrid between GMHT OSR and *B. rapa*. Some seedlings showed glyphosate resistance. These observations suggest that hybridisation between GMHT OSR and feral *B. rapa* has probably occurred spontaneously. Middle Europe is the centre of origin for OSR. Hence, several species of Brassicaceae which are sexually compatible with OSR exist in this area. In Austria, there are more than twenty related species present (PASCHER & GOLLMANN 1999). Hybridisation is a possible way for the introduction of transgenes into weedy populations which could lead to unintentional changes in natural species composition.

## Transgene persistence in OSR – the soil seedbank

Seed shattering before and during harvest is a typical feature for the crop OSR. The seeds are buried in the soil and can enter a secondary dormancy (LUTMAN et al. 2003, GRUBER et al. 2004). Seedlings will continue to emerge for many years from the soil seedbank (SQUIRE et al. 2011). Most of those dormant seeds emerge in the first four years. BEGG et al. (2008) counted 200 seeds/m<sup>2</sup> after three years of seed rain. However, it was shown that the seeds of the soil seedbank are in general able to emerge in the soil also ten years after burial (D'HERTEFELDT et al. 2008).

### Previous Austrian justification for the GM OSR import ban and previous scientific findings

The previous Austrian justification states that feral OSR populations are able to build up long persisting soil seedbanks. They are important for the dynamics of OSR

feral populations, with large seed input from fields and seed spillage during transport activities (PIVARD et al. 2007). The soil seedbank and its persistence were already investigated in several previous publications (e.g. COLBACH et al. 2008; DEBELIAK et al. 2008; GRUBER et al. 2010; ANDERSEN et al. 2010). In the following section new scientific findings are discussed.

### Recent scientific findings (2011-2012)

The soil seedbank is important for initialising feral OSR's emergence. The seedbank of OSR is very long-living. The persistence of OSR seeds in the soil is of particular importance and moreover, it is a good indicator for the potential of future volunteers and feral plants (MIDDELHOFF & al. 2011). The feral seedbank in principle consists of seeds intended for planting as well as commodities that can function biologically as seed introduced into the location from outside by transportation vehicles, road verge mowers, animals or by movement of soil for agricultural and building work and seeds from plants reproducing directly on the site (DEVOS et al. 2012).

In the model of REUTER et al. (2011) an initially high average content of GM seeds of 17.3% in the seedbank of an OSR field was calculated. After ten years it was reduced to 0.3%. The same decline was noticed in the GM admixture in the harvest crop, from 15.8% in the third year to an average value of 0.2 in the tenth year. The model shows that persistence of GM seeds in the seedbank is assumed to be problematic for the subsequent conventional OSR crops.

MUNIER et al. (2012) showed that volunteers from dormant OSR seeds produced thousands of plants per hectare in the fourth year following a crop harvest. The combination of dormant seeds and GM herbicide resistance make GMHT OSR a new weed in California which will be difficult to manage.

Repeated seed addition and redistribution from various sources are important factors for building up a stable soil seedbank. On a larger scale in the landscape feral OSR can be considered long-lived with a proportion of the populations founded by repeated seed spills from both agricultural fields and transport (DEVOS et al. 2012).

### **Relevance for supporting the Austrian import-bans – transgene persistence in OSR – the soil seed bank**

New scientific studies support the perception that OSR seeds can persist in a viable form in the soil for many years. Moreover, new evidence is given that regular anthropogenic disturbance like mowing or herbicide application supports the establishment and persistence of feral OSR. In Austria, verges of main roads are mowed regularly which is assumed to encourage the establishment of feral populations.

# Herbicide tolerance, gene stacking, and fitness

## Austrian justification for the GM OSR import ban and previous scientific findings

Gene flow among different GMHT OSR varieties can lead to the development of multiple HR OSR plants (e.g. KNISPEL et al. 2008). These multi-resistant plants can create management challenges for the farmers because sites which are sprayed with herbicides could provide locations which might favour feral GMHT OSR plants. Preferred habitats of feral OSR like railways are usually treated with glyphosate in Austria once a year (SATTELBERGER 2001).

## Recent scientific findings (2010-2012)

In general, GMHT OSR has the potential to become a **new glyphosate resistant weed** on roadsides, orchards, vineyards, fallow fields, and fields cultivated with glyphosate resistant crops because of its ability to produce a significant percentage of secondary dormant seeds thus reducing the effectiveness of glyphosate (MUNIER et al. 2012). Glyphosate is the mainly used herbicide in California where GMHT OSR is cultivated to a high extent. In this region, feral GM glyphosate-resistant OSR was at first observed on roadsides in 2009. Other glyphosate resistant crops – corn or cotton – have been widely cultivated in California over the past ten years but have not become established along roadsides as reproducing weeds. This example from a country growing GMHT OSR underlines that OSR is a competitive weed. Moreover, feral GMHT OSR plants have already emerged, which are resistant to both herbicides glyphosate and glufosinate. These plants might become a future problem for farmers because more effective herbicides will have to be used to be able to remove these plants.

Feral GMHT OSR and hybrids with compatible weedy species are able to grow outside cultivation on locations without herbicide spraying. Glyphosate exposure can extend beyond crop field boundaries. Herbicide drift could function as a selective agent contributing to **increased transgene persistence** in the environment. In the study of LONDO et al. (2010) a reduction in reproductive fitness could be shown for non-transgenic genotypes. **An increase in plant fitness was demonstrated for transgenic genotypes as a result of glyphosate-drift treatment.** Glyphosate drift may influence the movement of transgenes among transgenic crops and weeds. The study confirms the potential for persistence of glyphosate resistance transgenes in weedy plant communities due to the effect of glyphosate spray drift on plant fitness. Glyphosate drift has the potential to change gene-flow dynamics between compatible GM crops and weeds.

A typical roadside and field edge plant community was constructed containing three sexually compatible *Brassica* species and exposed to glyphosate drift in a study in the United States (WATRUD et al. 2011). After two years, changes were observed in community composition, plant density, and biomass in both control and treatment mesocosms. In the mesocosm exposed to glyphosate drift *Brassica* remained the dominant genus and the incidence of the herbicide resistance (CP4 EPSPS) transgene increased. From the results it is concluded that glyphosate drift can contribute to the persistence of *Brassica* that expresses the glyphosate resistance transgene.

CHIFFLET et al. (2011) address that feral OSR populations should be rigorously managed to eliminate relay points for pollen dispersal at the landscape scale. The idea sounds good but as feral OSR is widespread and not only present at road verges but also on riverbanks, field edges etc., an effective control will hardly be feasible, at least in the Austrian small scaled agricultural landscapes.

A study conducted in China tested the interspecific-hybrids produced between four *B. juncea* populations with different glyphosate-susceptibility and transgenic OSR (HUANGFU et al. 2011). Previous studies within the *Brassica* complex have shown that in some cases hybrid fitness was higher than that of both parents, in other cases it was the opposite. These variations in fitness reflect the magnitude of genetic differences. In this study the fitness-related components and photosynthetic capacity of hybrids decreased dramatically in comparison with both parents. **However, significant differences were observed among different hybrids.** All F1 hybrids exhibited highly enhanced but similar herbicide tolerance levels regardless of the wild *B. juncea* parent indicating that genetically engineered herbicide tolerance may over-dominate phenotype ones by wild-crop hybridisation (HUANGFU et al. 2011, page 57). It was observed that OSR genetic markers could be transferred at relatively high frequencies to the next generation. The findings imply that the resistant trait would be readily available under a new genetic background after hybridisation. The hybrids could then be favoured under selection. In this context it is important to consider not only the average fitness but also the differences among individuals.

## **Relevance for supporting the Austrian import-bans – herbicide tolerance, gene-stacking and fitness**

The discussed recent studies show that HT traits are able to spread in feral OSR plants outside cultivation. In contrast to the general assumption that **herbicide resistance is considered a neutral trait and does not alter the fitness of a plant** in an environment which is sprayed with a specific herbicide, the herbicide drift even in amounts much lower than the field application rate was shown to alter the fitness of GMHT OSR (LONDO et al. 2010; KIM 2012). Typical sites for the occurrence of OSR like roadside habitats might provide corridors for movement of GM pollen

and seeds into new areas and increase the potential for introgression of transgenes into plant communities as shown by WATRUD et al. (2011).

Contrary to OSR cultivation where the increased use of glyphosate over multiple years imposes selective genetic pressure on weed populations, usage of glyphosate outside of GMHT crops will not lead to the development of HT weeds so rapidly. The wide distribution of feral GMHT OSR in Austria, however, might enable that glyphosate resistant plants could colonise sites where no weed is expected to grow because of spraying, e. g. railways. A relevant adverse ecological effect would arise if the presence of such feral GMHT OSR plants on these sites required an increased herbicide application which would indirectly increase selection pressure on the plant community.

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Ziel dieses Gutachtens ist die Erbringung von neuen wissenschaftlichen Kenntnissen betreffend der potentiellen ökologischen Auswirkungen des Imports von gentechnisch veränderten Rapslinien als Grundlage für die Rechtfertigung der Verlängerung der österreichischen Importverbote für herbizidtoleranten Raps Ms8, Rf3 und Ms8xRf3 und GT73. Die Schlussfolgerungen dieses Gutachtens basieren hauptsächlich auf aktuellen wissenschaftlichen Kenntnissen, die zwischen 2011 und 2012 publiziert wurden. Aber auch die bisherigen wissenschaftlichen Rechtfertigungen, die von Österreich zur Untermauerung der Importverbote dieser GV Rapslinien vorgelegt wurden, werden weiterhin als gültig angesehen.