frontiers in Ecology and Evolution

PERSPECTIVE
published: 04 September 2017
doi: 10.3389/fevo.2017.00104

Spillage of Viable Seeds of Oilseed Rape along Transportation Routes: Ecological Risk Assessment and Perspectives on Management Efforts

Kathrin Pascher[1,2], Christa Hainz-Renetzeder[2,3], Günter Gollmann[4] and Gerald M. Schneeweiss[2]*

[1] Department of Integrative Biology and Biodiversity Research, University of Natural Resources and Life Sciences, Vienna, Vienna, Austria, [2] Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria, [3] Department of Landscape, Spatial and Infrastructure Sciences, University of Natural Resources and Life Sciences, Vienna, Vienna, Austria, [4] Department of Theoretical Biology, University of Vienna, Vienna, Austria

OPEN ACCESS

Edited by:
Clara Grilo, Federal University of Lavras, Brazil

Reviewed by:
Kelly Ksiazek-Mikenas, Northwestern University, United States
Anna L. Johnson, University of Maryland, Baltimore County, United States

***Correspondence:**
Kathrin Pascher
kathrin.pascher@boku.ac.at;
kathrin.pascher@univie.ac.at

Specialty section:
This article was submitted to Urban Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *18 March 2017*
Accepted: *18 August 2017*
Published: *04 September 2017*

Citation:
Pascher K, Hainz-Renetzeder C, Gollmann G and Schneeweiss GM (2017) Spillage of Viable Seeds of Oilseed Rape along Transportation Routes: Ecological Risk Assessment and Perspectives on Management Efforts. Front. Ecol. Evol. 5:104. doi: 10.3389/fevo.2017.00104

Seed spillage during handling and transportation promotes establishment and invasion of feral crops into adjacent semi-natural habitats. This is also the case for oilseed rape (OSR, *Brassica napus*), where seed spillage may lead to establishment of herbicide resistant OSR populations in countries without cultivation of genetically modified OSR. Using data from Austria—where cultivation and import of genetically modified OSR are banned—as a prime example, we demonstrate that ports, oil mills, switchyards, and border railway stations to countries with different electric current systems—where trains have to stop—are the sites of primary concern with respect to seed spillage. Based on the results of the Austrian case study we discuss common measures to limit crop seed spillage which include intensified controls at border railway stations and the mode of seed packing during transportation. We further recommend sufficient cleaning both of goods wagons and of loading areas of trucks and ships as well as an appropriate weed management.

Keywords: oilseed rape, transport, seed spillage, feral crop, invasion, mitigation measures, risk assessment, Austria

Seeds of arable crops are regularly spilled during transport and handling activities. These incidents cause intense management efforts and additional costs (Yoshimura et al., 2006). Moreover, the origin and establishment of feral populations along transportation routes contribute to the uncertainty concerning containment of genetically modified (GM) crops outside fields and could therefore interfere with a successful weed management. Here, we focus on oilseed rape (OSR, *Brassica napus*), a frequently spilled crop (Von der Lippe and Kowarik, 2007) with GM lines already in use, to identify spillage hot-spots due to transportation and handling, allowing us to develop perspectives on common management approaches.

Spillage of OSR seeds has intensively been studied worldwide (e.g., Schafer et al., 2011), which makes OSR a primary model system in this context. We chose Austria as study region because this small country is situated in the center of Europe rendering it a nodal point for traffic and international goods carriage.

OILSEED RAPE AS A MODEL SYSTEM FOR SEED SPILLAGE ALONG TRANSPORT ROUTES AND FOR THE MANAGEMENT OF FERAL CROP PLANTS

Oilseed rape is a globally widely grown crop of hybrid origin, descending from the parent species cabbage (*B. oleracea*) and turnip (*B. rapa*). It is unknown as a wild plant, but frequently occurs as a feral plant outside cultivation (Pessel et al., 2001). It is mainly pollinated by insects, more rarely by wind, facilitating hybridisation with related species (Chevré et al., 2004), potentially leading to transfer of transgenes into wild populations. For Austria, Pascher and Gollmann (1999) identified more than 20 species as potential hybridisation partners. Oilseed rape seeds are small (1.3–3.1 mm diameter: Rich, 1991) and remain germinable in the soil up to several years (Gruber et al., 2004). At present, GM OSR is widely cultivated in Canada (>90% of OSR cultivation estimated), USA, Chile, and Australia (James, 2015). In the European Union cultivation of GM OSR is currently not authorized, but several OSR lines (e.g., herbicide resistant GT73, MS8, RF3, MS8 × RF3) are admitted for import, processing and feed use.

Feral OSR plants may originate from seed banks at semi-natural sites due to local soil relocation, as a result of epizoochory—diaspores are dispersed *via* adhesion to animals (e.g., wild boars; Schmidt et al., 2004)—or due to seed spillage during transport and handling activities. Transport and handling have been identified as the main reasons for spillage of OSR (Crawley and Brown, 2004; Pivard et al., 2008) and are therefore the focus of the present work. In Central Europe, feral OSR plants are able to reproduce, and populations may persist for several years outside cultivation, especially along transportation routes such as railway lines or roads, as known for France (Pessel et al., 2001; Garnier et al., 2008; Pivard et al., 2008), Germany (Dietz-Pfeilstetter et al., 2006; Menzel, 2006; Elling et al., 2009; Middelhoff et al., 2009; Franzaring et al., 2016), the Netherlands (Tamis and de Jong, 2010), Great Britain (Crawley and Brown, 2004; Squire et al., 2010) and Austria (Pascher et al., 2000, 2006, 2010). In Switzerland, feral glyphosate resistant OSR (GT73) was identified on four of 79 sample sites (Schoenenberger and D'Andrea, 2012), although like in the European Union GT73 is not permitted for cultivation. These four sites were ports and railway stations on the borders to France and Italy. The feral plants probably originated from spillage of contaminated OSR seed from freight trains (Hecht et al., 2014; Schulze et al., 2014, 2015). Spillage of GM OSR seeds along transportation routes was also confirmed in Japan where cultivation of GM OSR is also prohibited (Saji et al., 2005; Kawata et al., 2009; Nishizawa et al., 2009, 2010; Aono et al., 2011; Mizuguti et al., 2011), the United States (in North Dakota 80% of feral OSR proved to be GM: Schafer et al., 2011; Sagers et al., 2012) and Canada (Yoshimura et al., 2006; Knispel et al., 2008; Beckie and Warwick, 2010; Knispel and McLachlan, 2010). Consequently, transportation and handling activities during import of GM OSR are considered the main cause of unintended occurrence and establishment of feral GM OSR in countries without GM OSR cultivation. In these countries, import restrictions have been put into place because introgression of herbicide resistant transgenes may cause problems with weed management and may negatively affect the integrity of genetic resources in wild relatives (Londo et al., 2010).

To illuminate this issue we assess the mid-term to long-term probability of spillage, establishment and distribution of imported viable OSR seeds, using field data collected along transportation routes and at loading and handling sites in Austria, where the import of GM OSR is banned because of ecological concerns (Pascher, 2011, 2012). Austria relies on import of OSR to meet the demands of the Austrian market. Most of the imported OSR seed originates from European countries (currently mainly Hungary, Serbia and Slovakia), smaller amounts are imported from Chile and New Zealand. Data concerning transportation activities of OSR seeds to and within Austria were, however, difficult to obtain or not made available to us. Seeds are imported as bulk mixtures complying with quality standards (e.g., oil content, low content of erucic acid and glucosinolates, absence of GM material) and thus usually without designation of defined OSR varieties (personal communication from managers of warehouses, ports, railway stations). Therefore, the identity of origin of OSR varieties imported to Austria is unknown. Furthermore, since 2012, OSR belongs to the goods category "other products of vegetable origin" and is no longer recorded separately, rendering traceability impossible. Oilseed rape seeds are transported on ship, trains, and trucks. Whereas, available data for transportation *via* ship are rather detailed, those concerning transport *via* railway and trucks are scanty. For instance, the route taken for transport on the road can be freely chosen by the truck-drivers. The biggest fraction of OSR seeds is transported on roads, also at the expense of the rail transport sector, which lost most of its market shares (information provided by the Austrian Federal Railways).

Case Study in Austria

Sixty observation sites in Austria were selected taking transport routes as well as handling and loading sites for OSR into account (a detailed description of the selection protocol is given in Pascher et al., 2016). These sites include predefined hotspots, where OSR seed spillage is expected to occur frequently due to handling activities (6 railway stations along the Austrian borders; 2 switchyards; 6 ports; 3 oil mills that import OSR; 1 processing company) and randomly selected locations where OSR seed spillage is possible (10 railway stations each within and outside OSR cultivation areas; 11 road sections each within and outside OSR cultivation areas). Surveys were carried out in spring and summer 2014 and 2015. At each observation site information such as population size, growing conditions and stage of maturity was recorded. The presence of feral OSR plants was confirmed along relevant transport routes of goods traffic (Pascher et al., 2016). At 44 out of 60 surveyed sites, feral OSR was registered in 2014 and/or in 2015 (**Figure 1**). These also included some sites outside OSR cultivation areas, where feral OSR is expected to have originated from seed spillage of imported OSR rather than from transport of OSR seeds harvested in Austria. Most of the populations were present in both years, indicating their persistence over years. The plants flowered, had already

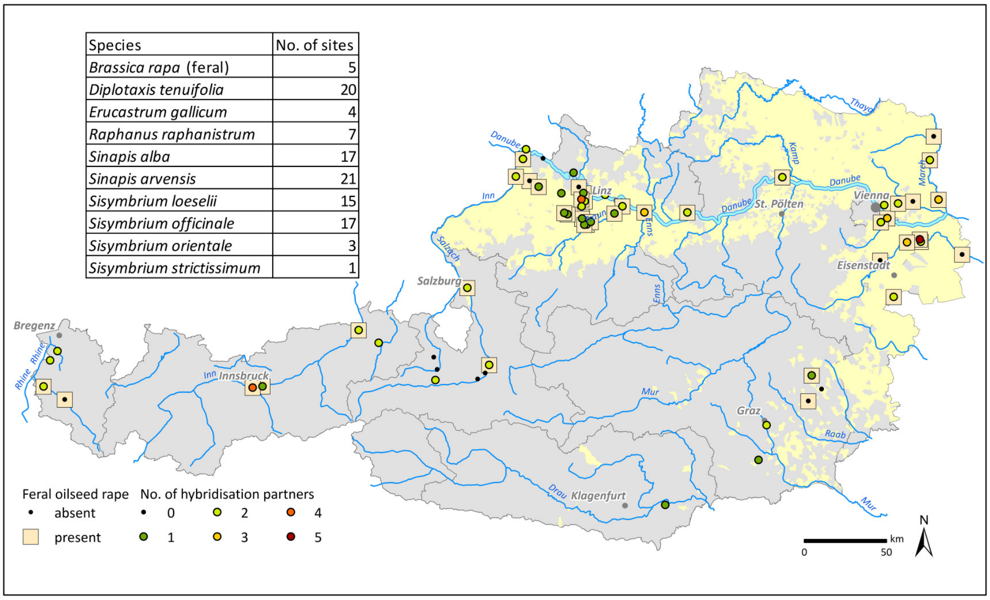

FIGURE 1 | Location of observation sites in Austria. The areas in light yellow indicate the oilseed rape (OSR) cultivation regions. Feral OSR (beige squares) was found at 44 of altogether 60 surveyed sites. Small and larger spots in different colors represent the number of one up to five different species of potential hybridisation partners of OSR registered at each of the 60 observation sites. The number of sites at which each of the 10 species was recorded is listed in the table.

developed viable seeds and exhibited high vitality. We found that the number of OSR plants was consistently higher in areas with cultivation than in those without (Wilcoxon-Mann-Whitney-Test: $p < 0.001$). At several surveyed sites feral OSR occurred in large numbers, e.g., up to 1,500 plants on a 2 km road section in Upper Austria, predominantly in outside curves, or thousands of individuals on the company premises of the largest Austrian OSR oil mill Bunge. Feral OSR was particularly common along railway lines. Among the 10 potential cross-breeding partners of OSR (Pascher et al., 2010) recorded at the 60 observation sites (**Figure 1**), *Sinapis arvensis* (at 21 sites) and *Diplotaxis tenuifolia* (at 20 sites) were found most frequently. In 25 sample sites, two species were registered, in one sample site even up to five potential hybridisation partners were found. The list of registered species in **Figure 1** may be incomplete, however, because at some surveyed sites observation had to focus on feral OSR only due to safety reasons and limited observation possibilities.

Identification of Most Sensitive Links

The most sensitive links in the transportation and processing chain for seed spillage of imported crops were identified to be spillage during transport and loading. Border railway stations, especially those where trains have to stop because of different electric current systems for running trains (e.g., between Italy and Austria), are hotspots for seed dispersal. Seed spillage along tracks may, however, generally increase, as controls of goods wagons (including checks whether the unloading hatches of the wagons were properly closed) are not made at intra-EU borders (i.e., Austrian border to Germany and Italy, and with the progressive extension of the EU, also to Czech Republic, Slovakia, Hungary and Slovenia). This is of particular concern, because Austria is connected to the railway-network of eight neighboring countries and more than 100 megatons of goods are transported here per year. Containment of truck loads is often inadequate, facilitating seeds spillage off the loading area especially in curves. Crop seeds such as oilseed rape are often transported unpacked on open loading areas of trucks and ships and goods carriage. Only occasionally, they are wrapped in sealed bags. Hence, seed spillage of OSR seeds can occur easily. Moreover, especially at ports in storage areas of unpacked grain, birds were observed to feed on the grain and could therefore pose an additional dispersal factor for OSR grain over longer distances (Wedlich et al., 2016). At locations where seeds are loaded and handled in a loose form, continuous seed spillage can be observed which is especially frequent with OSR. Hence, ports, switchyards, and OSR processing facilities and plants, such as oil mills are hotspots for seed spillage. Here, mixtures of OSR varieties as well as imported OSR seeds are handled. Schulze et al. (2014) confirmed GM OSR spillage at such sites in Switzerland. Although import of GM OSR seeds is banned, GM OSR was found in the St. Johann railway station and the Rhine port of Basel. Loading areas of trucks and ships as well as goods wagons are cleaned by the staff themselves. In general, cleaning is mandatory, but the method and the rigor are not defined. So, because of cursory and insufficient cleaning and small OSR seed size considerable amounts of seeds often remain on these transportation areas in spite of the cleaning (Pascher and Dolezel, 2005). Additionally, defect seals of trucks and goods wagons facilitate seed spillage. Additional hotspots for spillage are railway stations where defect trains are repaired. In the railway station of

Innsbruck we registered a large population of feral OSR on the stabling siding which probably originated from seed dispersal of a defect train.

Weed Management and Impurities in Imported Goods

If seeds have already reached the soil, proper weed management of feral plant populations is necessary to prevent persistence of feral plants. All tracks of the surveyed railway stations were sprayed using spraying wagons. The spraying train uses a detection system to recognize weeds along the tracks allowing selective and precise application of herbicides (pers. communication). Thus, it is possible to significantly reduce the amount of the sprayed herbicide. An additional way for seed dispersal is contamination in other goods. Wheat imports from Canada were identified as potential source for contamination with GM OSR seeds (GT73, MS8 × RF3, MS8, and RF3) in the Rhine port of Basel and in processing facilities of two grain mills in Switzerland (Schulze et al., 2015). Imported wheat, a main agricultural goods handled at the Rhine port of Basel, may contain a low level of impurities of GM OSR (impurity in wheat imported from Canada is estimated to be 0.005% at average). Although currently Austria has a high degree of self-supply of wheat (Pascher, 2013), accidental contamination of GM OSR in imported goods needs to be considered in the future.

PERSPECTIVES ON SAFEGUARD MITIGATION OF SEED SPILLAGE ON LONG-TERM BASIS

The establishment of feral crops such as OSR, especially of herbicide resistant lines, may contribute to the loss of biodiversity along transportation routes as well as at infrastructures in two ways. Firstly, feral OSR is able to form large populations, especially in disturbed and semi-natural habitats. Our study supports that some of these large populations establish in areas where no OSR is grown, which likely is due to import activities. Secondly, because of herbicide resistance of GM oilseed rape, these feral plants establish preferentially in habitats where herbicides are applied or that are affected by spraying in adjacent areas. Due to herbicide application selection pressure supports survival of feral glyphosate resistant plants, and this may lead to an introduction of transgenes into related species (Londo et al., 2010) growing in habitats that have to be kept free of vegetation. Consequently, sprayed railway tracks pose an appropriate habitat for the establishment of herbicide resistant OSR as it was shown in Switzerland (Schoenenberger and D'Andrea, 2012). Hence, in order to minimize and mitigate spillage of imported crops such as (GM) OSR, qualified measures need to be defined and implemented. First of all, the mode of seed packing during transportation plays a crucial role. In most cases OSR seeds are transported unpacked facilitating seed spillage. Spillage can be minimized by the use of sealed bags. The loading areas of ships and trucks should be checked regularly for perishing seals, in which case foam can be used to mend the holes. Moreover, at railway borders in Austria transportation facilities need to be checked for appropriate function, e.g., of unloading hatches. Likewise, applying intensified testing of grain cargos imported by ship might allow GM OSR contamination to be identified before unloading, handling and further transportation (Schulze et al., 2014). Cleaning of loading areas of trucks and ships, cargo boards of vehicles and train wagons, and storage areas at ports or oil mills should be intensified and performed carefully. Thereafter, these areas have to be checked for remaining OSR seeds. As seed spillage cannot be fully prevented, weed management has to be applied. Where possible, alternative weed management such as manual removal of plants (as currently already done in the surveyed Austrian ports), mowing, spraying of organic herbicides or grazing by animals is favored. In feral OSR populations spraying with herbicides may be considered to unmask GM OSR individuals, which then can be selectively removed. In contrast, in Switzerland the ports are sprayed and hence, long-term persistence of glyphosate resistant OSR seems to be very likely (Schulze et al., 2014). Weed control along railway lines is necessary to ensure worker safety as well as stability of railway gravel beds. In Austria, to maintain functionality of the beds, the gravel is regularly washed, reducing the amount of plant seeds in the material. Those measures, already performed for reasons of operational safety, would not be affected by the suggested additional weed management. The proposed safeguard measures that we consider necessary will, however, cause additional costs. The main responsibility to limit seed spillage involves the importers and traders, staff who handles and reloads the seeds as well as seed-processors.

To control appearance and persistence of feral crops along transport routes, a monitoring program for imported herbicide resistant crops such as OSR should be implemented in the future. In this context it needs to be considered that documentation of mode of transportation (ship, train, truck), transportation routes as well as loading and handling sites—so called commodity flows—is necessary to identify relevant sites for monitoring in each country and to implement a program taking local specificities into account. Relevant contact addresses (e.g., oil mills, ports) and sources (e.g., Statistik Austria, INVEKOS) for necessary information need to be provided in advance for an immediate update. For us, interviews with the staff of the contacted facilities have been particularly helpful to make domestic and foreign goods' transportation and handling more transparent. If monitoring of, for instance, OSR seed spillage during import activities were to be performed under financial and time constraints, monitoring should focus on reloading and handling sites of OSR in Austria, where the highest genetic diversity within populations of feral OSR plants, probably tracing back to repeated input of different OSR seeds also from abroad, has been found (Pascher et al., 2016). Besides the possibility to unmask herbicide resistant OSR, ecological concerns of the usage of herbicides exist concerning spraying of complementary herbicides, which could facilitate establishment of feral herbicide resistant OSR in case of GM OSR imports. Second in line for surveying are switchyards, transportation roads as well as railway lines leading to loading, handling and processing facilities. Analysing around 2,000 individual plants will provide comprehensive results for a genetic monitoring in small countries such as Austria (Pascher et al., 2016) or Switzerland (Hecht et al., 2014). This sample size is also financially feasible. Since single OSR varieties could not be characterized with eight SSR-markers (Pascher et al., 2016) a larger budget for genetic analyses must be provided to be able to enlarge the marker-set.

Safeguard mitigation efforts on long-term basis are in general needed to prevent establishment and spread of feral GM crops originating from seed spillage along transportation routes. Similar to OSR, the small seeds of cereals are also regularly spilled along roads (Von der Lippe and Kowarik, 2007) or along railway tracks (personal observation) resulting in numerous feral plants. As little documentation concerning cereal seed spillage exists, further detailed research is of utmost importance, in particular, because GM cereal lines have already been developed and could be in use in the near future. In order to succeed with weed management of feral crops along transportation routes, a broad collaboration both in research and practice for interdisciplinary exchange is necessary. Moreover, for a successful approach standards and international guidelines concerning transport of crops—as it would especially be necessary with OSR seeds—have to be harmonized in future.

AUTHOR CONTRIBUTIONS

KP conceived and designed the study and carried out field work. KP and CHR assembled agrarian data, information on transportation routes and import data. CHR carried out sampling and designed the figure. KP and GMS wrote the manuscript. GG commented it. All authors read and approved the final version of the manuscript before submission.

FUNDING

Funding was provided by the Austrian Federal Ministry of Health and Women's Affairs, Vienna (GZ 70420/0167-II/B/15/2013).

ACKNOWLEDGMENTS

We thank the staff of the Austrian Federal Railways OEBB, the sampled ports and oil processing facilities for their cooperativeness and kindness during our field visits. Many thanks to the organizers of the IENE 2016 Conference "Integrating Transport Infrastructure with Living Landscapes" in Lyon for granting the full publication fee of the present article.

REFERENCES

Aono, M., Wakiyama, S., Nagatsu, M., Kaneko, Y., Nishizawa, T., Nakajima, N., et al. (2011). Seeds of a possible natural hybrid between herbicide-resistant *Brassica napus* and *Brassica rapa* detected on a riverbank in Japan. *GM Crops* 2, 201–210. doi: 10.4161/gmcr.2.3.18931

Beckie, H. J., and Warwick, S. I. (2010). Persistence of an oilseed rape transgene in the environment. *Crop Protect.* 29, 509–512. doi: 10.1016/j.cropro.2009.11.013

Chevré, A. -M., Ammitzbøll, H., Breckling, B., Dietz-Pfeilstetter, A., Eber, F., Fargue, A., et al. (2004). "A review on interspecific gene flow from oilseed rape to wild relatives," in *Introgression from Genetically Modified plants into Wild relatives. Proceedings of the Congress "Introgression from Genetically Modified Plants into Wild Relatives and Its Consequences,"* eds H. C. M. den Nijs, D. Bartsch, and J. Sweet (Wallingford: Publishing Wallingford), 235–251.

Crawley, M. J., and Brown, S. L. (2004). Spatially structured population dynamics in feral oilseed rape. *Proc. R. Soc. Lond. B. Biol.* 271, 1909–1916. doi: 10.1098/rspb.2004.2814

Dietz-Pfeilstetter, A., Metge, K., Schönfeld, J., and Zwerger, P. (2006). Abschätzung der Ausbreitung von Transgenen aus Raps durch populationsdynamische und molekularbiologische Untersuchungen an Ruderalraps. *J. Plant Dis. Protect. Sonderheft Stuttgart*. 39–47.

Elling, B., Neuffer, B., and Bleeker, W. (2009). Sources of genetic diversity in feral oilseed rape (*Brassica napus*) populations. *Basic Appl. Ecol.* 10, 544–553. doi: 10.1016/j.baae.2009.01.005

Franzaring, J., Wedlich, K., Fangmeier, A., Eckert, S., Zipperle, J., Krah-Jentgens, I., et al. (2016). Exploratory study on the presence of GM oilseed rape near German oil mills. *Environ. Sci. Pollut. Res.* 23, 23300–23307. doi: 10.1007/s11356-016-7735-5

Garnier, A., Pivard, S., and Lecomte, J. (2008). Measuring and modelling anthropogenic secondary seed dispersal along roadverges for feral oilseed rape. *Basic Appl. Ecol.* 9, 533–541. doi: 10.1016/j.baae.2007.08.014

Gruber, S., Pekrun, C., and Claupein, W. (2004). Seed persistence of oilseed rape (*Brassica napus*): variation in transgenic and conventionally bred cultivars'. *J. Agric. Sci.* 142, 29–40. doi: 10.1017/S0021859604003892

Hecht, M., Oehen, B., Schulze, J., Brodmann, P., and Bagutti, C. (2014). Detection of feral GT73 transgenic oilseed rape (*Brassica napus*) along railway lines on entry routes to oilseed factories in Switzerland. *Environ. Sci. Pollut. Res.* 21, 1455–1465. doi: 10.1007/s11356-013-1881-9

James, C. (2015). *Global Status of Commercialized Biotech/GM Crops: 2015. ISAAA Brief No. 51.* Ithaca, NY: ISAAA.

Kawata, M., Murakami, K., and Ishikawa, T. (2009). Dispersal and persistence of genetically modified oilseed rape around Japanese harbors. *Environ. Sci. Pollut. Res.* 16, 120–126. doi: 10.1007/s11356-008-0074-4

Knispel, A. L., and McLachlan, S. M. (2010). Landscape-scale distribution and persistence of genetically modified oilseed rape (*Brassica napus*) in Manitoba, Canada. *Environ. Sci. Pollut. Res.* 17, 13–25. doi: 10.1007/s11356-009-0219-0

Knispel, A. L., McLachlan, S. M.,Van Acker, R. C., and Friesen, L. F. (2008). Gene flow and multiple herbicide resistance in escaped canola populations. *Weed Sci.* 56, 72–80. doi: 10.1614/WS-07-097.1

Londo, J. P., Bautista, N. S., Sagers, C. L., Lee, E. H., and Watrud, L. S. (2010). Glyphosate drift promotes changes in fitness and transgene flow in canola (*Brassica napus*) and hybrids. *Ann. Bot. Lond.* 106, 957–965. doi: 10.1093/aob/mcq190

Menzel, G. (2006). *Verbreitungsdynamik und Auskreuzungspotenzial von Brassica napus L. (Raps) im Großraum Bremen – Basiserhebung zum Monitoring von Umweltwirkungen transgener Kulturpflanzen.* Dissertation, Universität Bremen, Forschen und Wissen, GCA-Verlag, Waabs.

Middelhoff, U., Reiche, E. –W., and Windhorst, W. (2009). An integrative methodology to predict dispersal of genetically modified genotypes in oilseed rape at landscape-level – a study for the region of Schleswig-Holstein, Germany. *Ecol. Indic.* 11, 1000–1007. doi: 10.1016/j.ecolind.2009.03.008

Mizuguti, A., Yoshimura, Y., Shibaike, H., and Matsuo, K. (2011). Persistence of feral populations originated from spilled seeds around the Kashima seaport in Japan. *JARQ* 45, 181–185. doi: 10.6090/jarq.45.181

Nishizawa, T., Nakajima, N., Aono, M., Tamaoki, M., Kubo, A., and Saji, H. (2009). Monitoring the occurrence of genetically modified oilseed rape growing along a Japanese roadside: 3-year observations. *Environ. Biosaf. Res.* 8, 33–44. doi: 10.1051/ebr/2009001

Nishizawa, T., Tamaoki, M., Aono, M., Kubo, A., Saji, H., and Nakajima, N. (2010). Rapeseed species and environmental concerns related to loss of seeds of genetically modified oilseed rape in Japan. *GM Crops* 1, 143–156. doi: 10.4161/gmcr.1.3.12761

Pascher, K. (2011). *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 2008 to 2010.* Expert Report Commissioned by the Federal Ministry of Health, Vienna.

Pascher, K. (2012). *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*. Expert report commissioned by the Federal Ministry of Health, Vienna.

Pascher, K. (2013). *Ökologische Risikoabschätzung von Gentechnisch Verändertem Weizen – Identifizierung von Wesentlichen Aspekten und Konzeptentwicklung*. Expert report commissioned by the Federal Ministry of Health, Vienna.

Pascher, K., and Dolezel, M. (2005). *Koexistenz von Gentechnisch Veränderten, Konventionellen und Biologisch Angebauten Kulturpflanzen in der Österreichischen Landwirtschaft – Handlungsempfehlungen aus Ökologischer Sicht*. Wien: Im Auftrag des Bundesministeriums für Gesundheit und Frauen, Forschungsbericht der Sektion, IV, Band 2/2005.

Pascher, K., and Gollmann, G. (1999). Ecological risk assessment of transgenic plant releases: an Austrian perspective. *Biodivers. Conserv*. 8, 1139–1158. doi: 10.1023/A:1008904525713

Pascher, K., Hainz-Renetzeder, C., and Schneeweiss, G. M. (2016). *Risk of Seed Spillage of Imported Oilseed Rape Along Transport Routes – Assessment of potential Medium-Term to Long-Term Effects of an Accidental entry of Viable Seeds in Austria*. Study Report Commissioned by the Federal Ministry of Health and Women's Affairs. Avalialble online at: https://www.bmgf.gv.at/home/Gesundheit/Gentechnik/Fachinformation_Gruene_Gentechnik/Risk_of_seed_spillage_of_imported_oilseed_rape_along_transport_routes.

Pascher, K., Macalka-Kampfer, S., and Reiner, H. (2000). *Vegetationsökologische und Genetische Grundlagen für die Risikobeurteilung von Freisetzungen von transgenem Raps und Vorschläge für ein Monitoring*. Studie im Auftrag des Bundeskanzleramtes, Sektion, I. X., Bundesministerium für soziale Sicherheit und Generationen, Forschungsberichte.

Pascher, K., Macalka, S., Rau, D., Gollmann, G., Reiner, H., Glössl, J., et al. (2010). Molecular differentiation of commercial varieties and feral populations of oilseed rape (*Brassica napus* L.). *BMC Evol. Biol.* 10:63. doi: 10.1186/1471-2148-10-63

Pascher, K., Narendja, F., and Rau, D. (2006). *Feral Oilseed Rape – Investigations on its Potential for Hybridisation*. Study Report Commissioned by the Federal Ministry of Health and Women's Affairs, Vienna.

Pessel, F. D., Lecomte, J., Emeriau, V., Krouti, M., Messean, A., and Gouyon, P. H. (2001). Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. *Theor. Appl. Genet.* 102, 841–846. doi: 10.1007/s001220100583

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., et al. (2008). Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. *J. Appl. Ecol.* 45, 476–485. doi: 10.1111/j.1365-2664.2007.01358.x

Rich, T. C. G. (1991). *Crucifers of Great Britain and Ireland. BSBI Handbook,* Vol. 6, London: Botanical Society of the British Isles.

Sagers, C., Schafer, M., Murdoch, B., Londo, J., Travers, S., and van de Water, P. (2012). "Domestication, feral species and the importance of industrial agriculture to the future of plant diversity," in *Abstractbook of the International Conference "Implications of GM-crop Cultivation at Large Spatial Scales (GMLS 2012)*," (Bremen).

Saji, H., Nakajima, N., Aono, M., Tamaoki, M., Kubo, K., Wakiyama, S., et al. (2005). Monitoring the escape of transgenic oilseed rape around Japanese ports and roadsides. *Environ. Biosafety Res.* 4, 217–222. doi: 10.1051/ebr:2006003

Schafer, M. G., Ross, A. A., Londo, J. P., Burdick, C. A., Lee, E. H., Travers, S. E., et al. (2011). The establishment of genetically engineered canola populations in the U.S. *PLoS ONE* 6:e25736. doi: 10.1371/journal.pone.0025736

Schmidt, M., Sommer, K., Kriebitzsch, W.-U., Ellenberg, H., and von Oheimb, G. (2004). Dispersal of vascular plants by game in the northern Germany. Part I: Roe deer (Capreolus capreolus) and wild boar (Sus scrofa). *Eur. J. Forest Res.* 123, 167–176. doi: 10.1007/s10342-004-0029-3

Schoenenberger, N., and D'Andrea, L. (2012). Surveying the occurrence of subspontaneous glyphosate-tolerant genetically engineered *Brassica napus* L. *(Brassicaceae)* along Swiss railways. *Environ. Sci. Eur.* 24:23. doi: 10.1186/2190-4715-24-23

Schulze, J., Brodmann, P., Oehen, B., and Bagutti, C. (2015). Low level impurities in imported wheat are a likely source of feral transgenic oilseed rape (*Brassica napus* L.) in Switzerland. *Environ. Sci. Pollut. Res*. 22, 16936–16942. doi: 10.1007/s11356-015-4903-y

Schulze, J., Frauenknecht, T., Brodmann, P., and Bagutti, C. (2014). Unexpected diversity of feral genetically modified oilseed rape (*Brassica napus* L.) despite a cultivation and import ban in Switzerland. *PLoS ONE* 9:e114477. doi: 10.1371/journal.pone.0114477

Squire, R. G., Breckling, B., Dietz-Pfeilstetter, A., Jørgensen, R. B., Lecomte, J., Pivard, S., et al. (2010). Status of feral oilseed rape in Europe: its minor role as a GM impurity and its potential as a reservoir of transgene persistence. *Environ. Sci. Pollut. Res.* 18, 111–115. doi: 10.1007/s11356-010-0376-1

Tamis, W. L. M., and de Jong, T. J. (2010). *Transport Chains and Seed Spillage of Potential GM Crops with Wild Relatives in the Netherlands*. COGEM Report: CGM 2010-02. Available online at: http://cogem.net/index.cfm/en/publications/publicatie/transport-chains-and-seed-spillage-of-potential-gm-crops-with-relatives-in-te-netherlands

Von der Lippe, M., and Kowarik, I. (2007). Crop seed spillage along roads: a factor of uncertainty in the containment of GMO. *Ecography* 30, 483–490. doi: 10.1111/j.0906-7590.2007.05072.x

Wedlich, K. V., Franzaring, J., and Fangmeier, A. (2016). Wedlich, K. V., Franzaring, J., and Fangmeier, A. (2016). *Entwicklung und Erprobung eines Konzepts für ein Monitoring von für den Import zugelassenem transgenem Raps nach Richtlinie 2001/18/EG, Ergebnis eines F+E-Vorhabens (FKZ 3511 89 0100) des Bundesamtes für Naturschutz*. Bonn: BfN-Skripten 430.

Yoshimura, Y., Beckie, H. J., and Matsuo, K. (2006). Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. *Environ. Biosaf. Res.* 5, 67–75. doi: 10.1051/ebr:2006019

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.