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RESEARCH ARTICLE

Low level impurities in imported wheat are a likely source of feral transgenic oilseed rape (*Brassica napus* L.) in Switzerland

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Abstract In Switzerland, the cultivation of genetically modified (GM) oilseed rape (*Brassica napus* L.) and the use of its seeds for food and feed are not permitted. Nevertheless, the GM oilseed rape events GT73, MS8×RF3, MS8 and RF3 have recently been found in the Rhine port of Basel, Switzerland. The sources of GM oilseed rape seeds have been unknown. The main agricultural good being imported at the Rhine port of Basel is wheat and from 2010 to 2013, 19 % of all Swiss wheat imports originated from Canada. As over 90 % of all oilseed rape grown in Canada is GM, we hypothesised that imports of Canadian wheat may contain low level impurities of GM oilseed rape. Therefore, waste fraction samples gathered during the mechanical cleaning of Canadian wheat from two Swiss grain mills were analysed by separating oilseed rape seeds from waste fraction samples and testing DNA of pooled seeds for the presence of transgenes by real-time PCR. Furthermore, oilseed rape seeds from each grain mill were sown in a germination experiment, and seedling DNA was tested for the presence of transgenes by real-time PCR. GT73, MS8×RF3, MS8 and RF3 oilseed rape was detected among seed samples and seedlings of both grain mills. Based on this data, we projected a mean proportion of 0.005 % of oilseed rape in wheat imported from Canada. Besides Canadian wheat, the Rhine port of Basel does not import any other significant amounts of agricultural products from GM oilseed rape producing countries. We therefore conclude that Canadian wheat is the major source of unintended introduction of GM oilseed rape seeds into Switzerland.

Keywords Oilseed rape · *Brassica napus* · Genetically modified plants · Admixture · Impurities · Durum wheat · Cereal grains · Seed spillage · Transport · Environmental monitoring

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Introduction

Genetically modified (GM) oilseed rape (OSR, *Brassica napus* L.) has been adopted on a large scale in North and South America and Australia (James 2014). However, the cultivation of GM OSR is controversial in many countries, especially in Europe. Concerns focus on the high potential of GM OSR to outcross with conventional OSR (Damgaard and Kjellsson 2005; Hüsken and Dietz-Pfeilstetter 2007) and with related species (Chèvre et al. 2004; Liu et al. 2013) which could lead to gene flow of transgenes to conventional crops or wild species, respectively, and the potential of OSR to persist as volunteer plants in agricultural fields (D'Hertefeldt et al. 2008; Jørgensen et al. 2007) or as feral plants in the environment (Elling et al. 2009; Pascher et al. 2010). In Switzerland, the use of GM OSR seeds for food and feed is not authorised (FASC 2005, 2014), and a moratorium for the cultivation of GM crops was put into force in 2005 (FASC 2003). This moratorium will last at least until the end of 2017 (FASC 2003), and the control and monitoring of feral GM plants is mandatory (FASC 2008). Nevertheless, feral GM OSR has been found repeatedly in Switzerland (Hecht et al. 2014; Schoenenberger and D'Andrea 2012; Schulze et al. 2014). In the most recent study, the occurrence of feral herbicide resistant GM OSR events GT73 (Roundup Ready, Monsanto), MS8×RF3, MS8 and RF3 (all traded as InVigor, Bayer

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CropScience) has been reported for the Rhine port of Basel in Switzerland (Schulze et al. 2014). These GM OSR events contain transgenes conferring resistance against the herbicides glyphosate (GT73) or glufosinate (MS8×RF3, MS8, RF3). Highest densities of GM OSR plants were found at unloading sites for ships, and a few plants grew along outbound railway lines and roads. Given the existing Swiss regulations, the diversity of detected GM OSR events in the Rhine port of Basel was unexpected (Schulze et al. 2014). To date, the sources of GM OSR seeds have not been determined. Possible sources for an unintended introduction of GM OSR are impurities of imports of OSR seeds or other agricultural products. GM OSR is not tolerated in Swiss food (FASC 2005; FSVO 2013), but in Swiss feed, adventitious presence of GM OSR can be tolerated up to a proportion of 0.5 % (FASC 2011) subject to approval of the GM OSR event by the responsible authority. Therefore, the regular import of feed containing small amounts of viable GM OSR could have led to the observed distribution of feral GM OSR in the Rhine port of Basel. Enquiries showed that OSR imports at the Rhine port of Basel consisted mainly of crushed OSR from 2010 to 2014 (personal communication, Port of Switzerland). Germinable OSR seeds were only imported sporadically and in relatively small volumes, but no detailed data on OSR seed imports could be provided. However, all OSR import goods originated from Europe. The GT73, MS8×RF3, MS8 and RF3 OSR events have received authorization for food and feed use in the European Union, but there is no authorization for cultivation (European Commission 2015). Therefore, it is highly unlikely that GM OSR seeds have been introduced via imported OSR. Regarding other agricultural products, wheat comprising common wheat (*Triticum aestivum* L.) and durum wheat (*T. durum* Desf.) is the most important commodity in the Rhine port of Basel. Between 2010 and 2013, a total of 530'000 t of imported wheat was handled in the Rhine port of Basel (compiled from Port of Switzerland 2015), accounting for 33 % of Swiss wheat imports (SBV 2011, 2012, 2013, 2014). During the same period, Swiss annual imports of Canadian wheat averaged 77,512 t, representing 19 % of total wheat imports (SBV 2011, 2012, 2013, 2014). Under current agricultural practices in Canada, Canadian wheat is a likely source for the introduction of GM OSR seeds. Since 2009, GM OSR acreage always exceeded 90 % of the total OSR crop acreage in Canada (James 2010, 2011, 2012, 2013, 2014). Consequently, Canadian wheat may contain low level impurities of GM OSR seeds.

To assess the potential role of Canadian wheat as a pathway for the introduction of GM OSR into Switzerland, we tested Canadian durum wheat samples for the presence of GM OSR seeds. To this end, we received waste fraction samples gathered during the cleaning of Canadian durum wheat by two Swiss grain mills. Samples were searched for OSR seeds of which subsamples were tested for germination capacity. DNA of seedlings and seeds was tested for the presence of molecular markers of genetical modifications.

Materials and methods

Sampling and separation of adventitious OSR seeds

We received samples of waste fractions of Canadian durum wheat gathered during mechanical cleaning by the grain mills of Jowa (Wildegg; hereafter called grain mill 1) and Swissmill (Zurich; hereafter called grain mill 2). Both grain mills import their Canadian durum wheat through the Rhine port of Basel. Upon arrival at grain mills, wheat is routinely cleaned from impurities by sieving through different mesh sizes. The sieved waste is either used for biogas production or crushed and processed for feed (personal communication, Jowa and Swissmill). In a first step, we obtained waste fraction samples from different sieving steps and determined the waste fraction which contained the most OSR seeds (data not shown). From this fraction, each grain mill collected ten samples on ten different days in 2014. Samples weighed between 308 and 622 g. Grain mill 1 collected samples from March 19th to April 16th, and we received them on April 17th. Grain mill 2 collected samples from March 27th to May 14th, and we received them on June 6th. From each sample, all OSR seeds were sorted out manually with forceps and weighed to calculate the mass proportion per sample. OSR seeds were identified on the basis of seed shape, colour and size. Seeds of OSR are difficult to distinguish from seeds of the closely related *B. rapa* L. and *B. juncea* (L.) Czern (Baxter and Copeland 2008), which are also cultivated for oilseed production in Canada (Canola Council of Canada 2014a). Therefore, a part of seeds identified as OSR may be *B. rapa* or *B. juncea*.

Projection of total impurity

Mass proportions of OSR seeds were calculated in each waste fraction sample (mp1) for grain mill 1 and 2:

$$\mathrm{mp1} = \frac{\mathrm{mass\ (OSR\ seeds)}}{\mathrm{mass\ (waste\ fraction\ sample)}}$$

Grain mill 1 provided us with the mass proportions of the total waste fractions, from which samples were taken, in the total wheat batch prior to cleaning (mp2):

$$\mathrm{mp2} = \frac{\mathrm{mass\ (total\ waste\ fraction)}}{\mathrm{mass\ (total\ wheat\ batch)}}$$

This allowed for a projection of the impurity of the total wheat batches of grain mill 1 with OSR seeds by multiplication of mp1 and mp2:

Proportion of OSR seeds in total wheat batch = mp1 * mp2

Grain mill 2 could not provide mass data for the calculation of mp2.

Extraction of seed DNA

A random pool sample of ten OSR seeds was collected from each of the ten waste fraction samples per grain mill. Pool samples of seeds were ground to powder in liquid nitrogen using mortar and pestle. DNA was extracted from ground powder with the DNeasy Plant Mini Kit (Qiagen, Switzerland) in combination with the extraction robot QIAcube (Qiagen, Switzerland) according to the manufacturer's standard protocol. DNA concentration was measured at 260 nm with a spectrophotometer (Nanodrop ND-1000, Fisher Scientific AG, Switzerland) and diluted to a concentration of 2 ng μl^{-1} for real-time PCR.

Germination experiment and extraction of seedling DNA

We randomly sorted out 50 OSR seeds from each of three waste fraction samples per grain mill, resulting in a total of 150 seeds per grain mill (hereafter referred to as seed group 1 and 2). The three waste fraction samples per grain mill consisted of the first, the fifth and the tenth sample within the sampling series. To break possible dormancy, seeds were soaked in 0.5 % potassium nitrate for 24 h. The two seed groups were then planted separately into two trays (L×W×H: 21×15×4 cm) filled with finely sieved soil. Trays were kept at room temperature, and soil was regularly moistened with tap water. Samples from the two grain mills were received at different times, and the separated seeds were sown as soon as possible. Seed group 1 and seed group 2 were sown on June 4th and July 11th, respectively. Plant germination was observed for 4 weeks. Germinated plants were harvested at the cotyledon stage and placed in 1.5-ml tubes (Eppendorf, Switzerland). Tubes containing plants were dipped in liquid nitrogen, and the frozen plants were ground to powder within the tube with a plastic pestle on ice. DNA was extracted from ground powder and prepared for real-time PCR as described above.

Real-time PCR for amplification of genetic modification markers

All DNA samples were amplified and analysed in duplicate on a Rotor-Gene Q real-time PCR cycler (Qiagen, Switzerland) under the following cycling conditions: initial heating for 15 min at 95 °C followed by 45 cycles of amplification of 15 s at 95 °C and 60 s at 60 °C. Qualitative real-time PCR was carried out in 15 μl reaction volume containing 1x QuantiTect Multiplex PCR Master Mix (Qiagen, Switzerland), 6 ng template DNA, and the primers and probes specified in Table 1. DNA samples were first amplified with primers and probes for the plant-specific actin gene and the false negative control (FNC) fragment in a duplex real-time PCR to test for DNA quality and PCR inhibiting agents. Multiplex real-time PCR was then carried out to test for the following: (1) the phosphinotricin acetyltransferase genes bar and pat (conferring a resistance against the herbicide glufosinate), the glyphosate oxidoreductase gene gox and the enolpyruvylshikimate-3-phosphate synthase gene CP4 epsps (both conferring glyphosate-resistance) in a tetraplex real-time PCR, (2) the cauliflower mosaic virus 35S promoter (35S-P) and the *A. tumefaciens* nopaline synthase terminator (NOS-T) in a duplex real-time PCR. Singleplex real-time PCR was carried out to test for the event-specific sequences of GT73 (Roundup Ready, Monsanto), MS1 (InVigor, Bayer), MS8 (InVigor, Bayer), RF1 (InVigor, Bayer), RF2 (InVigor, Bayer), RF3 (InVigor, Bayer) and 73496 (Optimum, DuPont). All primers and probes were purchased from Eurogentec (Belgium) except primers and probes for the actin gene and the FNC fragment, which were purchased from Microsynth (Switzerland).

Reference DNA

As positive control for real-time PCR, reference plasmids were used containing the target sequences for the detection of either actin, bar, pat, gox and CP4 epsps or 35S-P and NOS-T as described by Hecht et al. (2014). A plasmid containing a random synthetic DNA sequence of 111 bp length served as FNC (Hecht et al. 2014). Certified reference material of the transgenic OSR events GT73, MS1, MS8, RF1, RF2 and RF3 was purchased from AOCS (USA). Certified reference material of the transgenic OSR event 73496 was purchased from the European Commission Joint Research Centre (Belgium).

Results

Projection of total impurity

Waste fraction samples of Canadian durum wheat consisted mainly of wheat fragments. However, all samples contained small amounts of intact OSR seeds. Mean mass proportions *mp1* of OSR seeds in waste fraction samples were 2.8 % (±0.8 % SD) and 1.9 % (±1.3 % SD) for samples of grain mill 1 and 2, respectively. The mean proportion of OSR seeds in the total wheat batches of grain mill 1 was projected as 0.005 % (±0.002 % SD) prior to cleaning.

Germination experiment and seedling analysis

A total of 24 and 51 seedlings were germinated and were harvested from seed groups 1 and 2, respectively (Table 2a).

Table 1 Specifications of primer and probe systems

Gene or target sequence	Oligonucleotide (final PCR concentration)	Name	Sequence 5′-3′[a]	Reference
actin	Forward primer (0.13 μM)	act-f	CAA GCA GCA TGA AGA TCA AGG T	Laube et al. 2010
	Reverse primer (0.13 μM)	act-r	ACA ATC TGT TGG AAA GTG CT GAG	
	Probe (0.035 μM)	act-p	ROX-CCT CCA ATC CAG ACA CTG TAC TTY CTC TC-BHQ2	
False negative control	Forward primer (0.13 μM)	fnc-f	CGT CAC ATC GGT AGA CGA ACT AA	Hecht et al. 2014
	Reverse primer (0.13 μM)	fnc-r	TTC AAG TCC TGA GCG GTT GTA A	
	Probe (0.035 μM)	fnc-p	JOE-ACC TAA CGC AGC AAC TTA TCG ACC GTT CAC TT-BHQ1	
35S-P	Forward primer (0.64 μM)	35S-f	GCC TCT GCC GAC AGT GGT	FASC 2001
	Reverse primer (0.64 μM)	35S-r	AAG ACG TGG TTG GAA CGT CTT C	
	Probe (0.16 μM)	35S-p	FAM-CAA AGA TGG ACC CCC ACC CAC G-BHQ1	
NOS-T	Forward primer (0.64 μM)	NOS-f	ATG ACG TTA TTT ATG AGA TGG GTT TTT A	FASC 2001
	Reverse primer (0.64 μM)	NOS-r	TTG CGC GCT ATA TTT TGT TTT C	
	Probe (0.16 μM)	NOS-p	YY-AGA GTC CCG CAA TTA TAC ATT TAA TAC GCG A-BHQ1	
bar	Forward primer (0.4 μM)	bar-f	CTG CAC CAT CGT CAA CCA CTA C	Hecht et al. 2014
	Reverse primer (0.4 μM)	bar-r	GAT AGC GCT CCC GCA GAC	
	Probe (0.2 μM)	bar-p	FAM-CGT ACC GAG CCG CAG GAA CCG CAG GAG T-BHQ1	
pat	Forward primer (0.5 μM)	pat-f	CGC GGT TTG TGA TAT CGT TAA C	Zeitler et al. 2002
	Reverse primer (0.5 μM)	pat-r	TCT TGC AAC CTC TCT AGA TCA TCA A	
	Probe (0.2 μM)	pat-p	CY5-AGG ACA GAG CCA CAA ACA CCA CAA GAG TG-BHQ2	
CP4 epsps	Forward primer (0.6 μM)	epsps-f	CCA ATG GGT CGT GTG TTG AA	Zeitler et al. 2002
	Reverse primer (0.6 μM)	epsps-r	TTG GCG TTG GAG TCT TTG GT	
	Probe (0.2 μM)	epsps-p	JOE-AGA CGG TGA TCG TCT TCC AGT TAC CTT GC-BHQ1	
gox	Forward primer (0.5 μM)	gox-f	CCG TGG AGG TTG GGA ACT T	Hecht et al. 2014
	Reverse primer (0.5 μM)	gox-r	CCC TTG GTA AAG GCG TGA GA	
	Probe (0.2 μM)	gox-p	ROX-CTG ATG CAT TGC GTG ATT TCG ATC CTA AC-BHQ2	
GT73	Forward primer (0.3 μM)	GT73F1	TCA TAC TCA TTG CTG ATC CAT GTA GA	Hecht et al. 2014
	Reverse primer (0.9 μM)	GT73R1	AAG CTT ATA CGA AGG CAA GAA AAG G	
	Probe (0.2 μM)	GT73TMP1	FAM-TTC CCG GAC ATG AAG ATC ATC CTC CTT C-DABCYL	
MS8	Forward primer (0.4 μM)	KVM085	GTT AGA AAA AGT AAA CAA TTA ATA TAG CCG G	Mazzara et al. 2007
	Reverse primer (0.4 μM)	HCA048	GGA GGG TGT TTT TGG TTA TC	
	Probe (0.2 μM)	TM011	FAM-AAT ATA ATC GAC GGA TCC CCG GGA ATT C-TAMRA	
MS1	Forward primer (0.4 μM)	MLD025	ACG CTG CGG ACA TCT ACA TT	Mazzara 2011a
	Reverse primer (0.4 μM)	MDB175	CTA GAT CGG AAG CTG AAG ATG G	
	Probe (0.2 μM)	TM030	FAM-CTC ATT GCT GAT CCA CCT AGC CGA CTT-TAMRA	
RF3	Forward primer (0.4 μM)	KVM084	AGC ATT TAG CAT GTA CCA TCA GAC A	Mazzara et al. 2007
	Reverse primer (0.4 μM)	DPA165	CAT AAA GGA AGA TGG AGA CTT GAG	
	Probe (0.2 μM)	TM010	FAM-CGC ACG CTT ATC GAC CAT AAG CCC A-TAMRA	
RF2	Forward primer (0.4 μM)	MDB207	GGG TGA GAC AAT ATA TCG ACG	Mazzara 2011c
	Reverse primer (0.4 μM)	KVM171	GGG CAT CGC ACC GGT GAG	
	Probe (0.2 μM)	TM024	FAM-CAC CGG CCA AAT TCG CTC TTA GCC GT-TAMRA	
RF1	Forward primer (0.4 μM)	MDB118	CTA AGG GAG GTC AAG ATG TAG C	Mazzara 2011b
	Reverse primer (0.4 μM)	KVM170	CGG GCC TAA CTT TTG GTG TG	

Table 1 (continued)

Gene or target sequence	Oligonucleotide (final PCR concentration)	Name	Sequence 5′-3′[a]	Reference
	Probe (0.2 μM)	TM022	FAM-CTC ATC ATC CTC ACC CAG TCA GCA TCA-TAMRA	
73496	Forward primer (0.6 μM)	73496-f	TCT CTT CAT AGC TCA TTA CAG TTT T	Based on Jacchia et al. 2014
	Reverse primer (0.6 μM)	73496-r	CCT CCA TAG AGT TCA ACA TCT TAA	
	Probe (0.25 μM)	73496-p	FAM-TTA+GTT A+GA TCA+GGA TAT T+CT T+G-MGBNFQ	

[a] Locked nucleic acids base symbols : +A, +C, +G and +T

Germination rates therefore were 16 % for seed group 1 and 34 % for seed group 2. Primary dormancy is very low in OSR seeds and lost during storage (Gruber et al. 2004), and seeds were subjected to a dormancy breaking treatment. Therefore, all ungerminated seeds were rated dead. Among seedlings of both seed groups, we identified the GM OSR events GT73, MS8×RF3, MS8 or RF3 by real-time PCR (Table 2a). All seedlings that were tested positive for the event-specific sequence of GT73 gave a positive result for the herbicide resistance genes CP4 epsps and gox in the real-time PCR. All seedlings that were tested positive for either the event-specific sequence of MS8 or RF3 or both of them (MS8×RF3) gave a positive result for the herbicide resistance gene bar and the NOS-T in the real-time PCR. The proportion of GM OSR seedlings among all seedlings was 67 % and 47 % for seed groups 1 and 2, respectively.

Seed analysis

The analysis of pool samples of seeds yielded identical results for the two grain mills. Among pool samples of seeds of both mills, the GM OSR event GT73 was identified in nine out of ten samples by real-time PCR (Table 2b). All pool samples of seeds that were tested positive for the event-specific sequence of GT73 gave a positive result for the herbicide resistance genes CP4 epsps and gox in the real-time PCR. Furthermore, in all ten pool samples of seeds of both mills, the GM OSR events MS8 and RF3 were identified by real-time PCR (Table 2b). MS8×RF3 is a hybrid variety derived from crosses between MS8 and RF3. Based on event-specific PCR methods for the identification of the MS8 and RF3 events, it cannot be concluded whether pool samples of seeds contained seeds that were homozygous or heterozygous for MS8 and RF3. All pool samples of seeds that were tested positive for the event-specific sequences of MS8 and RF3 gave a positive result for the herbicide resistance gene bar and the NOS-T in the real-time PCR.

Discussion

We could demonstrate consistent adventitious presence of the GM OSR events GT73, MS8×RF3, MS8 and RF3 in durum wheat imported from Canada by two Swiss grain mills. OSR impurities of Canadian durum wheat can be explained by

Table 2 **a** Numbers of seedlings of genetically modified oilseed rape (OSR) events GT73, MS8×RF3, MS8, RF3 and non-GM seedlings grown from OSR seeds gathered from Canadian wheat. **b** Results of analyses of pool samples of OSR seeds (pool samples consisted of ten seeds each) gathered from Canadian wheat. Samples of Canadian wheat were provided by two Swiss grain mills

a

Grain mill	No. of seeds sown	No. of seedlings germinated	Germination rate (%)	No. of seedlings of					Proportion of GM seedlings (%)
				GT73	MS8×RF3	MS8	RF3	non-GM	
1	150	24	16	3	5	4	4	8	67
2	150	51	34	7	7	5	5	27	47

b

Grain mill	No. of pool samples of seeds analysed	No. of pool samples of seeds containing		
		GT73	MS8[a]	RF3[a]
1	10	9	10	10
2	10	9	10	10

[a] MS8×RF3 is derived from crosses between the events MS8 and RF3. Therefore, proportions of seeds homozygous or heterozygous for these events cannot be deduced for pool samples of seeds

current agricultural practices. Rotation of cereal crops and OSR is a recommended practice to prevent the establishment of plant pests, as cereals and OSR share no diseases (Canola Council of Canada 2014b). Given the high potential of OSR to produce volunteer plants in subsequent crops (Lawson et al. 2006; Weber et al. 2014), low level impurities of wheat crops with seeds of volunteer OSR are possible. Alternatively, impurities may be added to agricultural goods during transport, storage or processing (Demeke et al. 2006).

The results of the seedling analysis suggest that a large proportion of OSR seeds in Canadian durum wheat is GM and at least partly viable. The total Swiss imports of Canadian wheat averaged 77,512 t per year between 2010 and 2013, but the available import data do not list durum and common wheat separately (SBV 2011, 2012, 2013, 2014). Assuming a similar impurity rate of 0.005 % for common and durum wheat, the OSR seed proportion in the annual Swiss imports of Canadian wheat would have amounted to 3.9 t. Based on a thousand seed weight of 3.2 g (Tamis and deJong 2009), this equates to 1.2 billion of OSR seeds. Multiplying this number with the seed germination rates (16–34 %) and the proportions of GM OSR seedlings (47–67 %) determined in the germination experiment results in a minimum and a maximum value of 90 and 273 million of germinable GM OSR seeds, respectively. Therefore, Canadian wheat must be regarded as a relevant source of introduction of GM OSR seeds into Switzerland, particularly concerning the large import volumes. Besides Canadian wheat, we are not aware of any other agricultural products of GM OSR producing countries that are imported in the Rhine port of Basel in significant quantities. Therefore, our results point to Canadian wheat as the major, if not the only, source of GM OSR seeds in Switzerland. This conclusion is further supported by the fact that all GM OSR events that were previously found in the Rhine port of Basel (Schulze et al. 2014) were also detected in the present study.

It is striking that GM OSR impurities of Swiss durum wheat imports have not been detected so far. This is likely because imported wheat is only occasionally tested for adventitious presence of GM material as there are no GM wheat cultivars on the market. Furthermore, cleaning at grain mills effectively removes most, if not all, adventitious GM OSR seeds from wheat. Thus, any small remaining amounts of GM OSR are most probably not detectable in products processed from Canadian wheat. The projected impurity rate of 0.005 % of OSR in durum wheat may underestimate the actual impurity, because only samples of the sieved waste fraction containing the most OSR seeds were analysed. Other waste fractions also contain small amounts of OSR seeds. However, even assuming an error of one order of magnitude and all contaminant OSR seeds being GM, our projected impurity rate would still be below the limit of reliable detection of standard PCR tests. Limits of detection of admixture of GM materials are dependent on the target crop and the test matrix. Most methodological studies found that GM materials can reliably be detected down to a level of about 0.1 %, with some exceptions below that level (Demeke and Perry 2014). The European Union Reference Laboratory for GM Food and Feed considers 0.1 % to be the lowest level at which results are satisfactorily reproducible between official laboratories (European Commission 2011). Therefore, GM OSR impurities at the low level found in this study are likely to remain undetected. However, as the example of the Rhine port of Basel shows, also low level GM impurities below the current limits of detection may be of concern regarding the accidental introduction of GM plants into the environment. To reduce the risk of future introductions of GM OSR seeds in Switzerland and especially in the Rhine port of Basel, efforts should focus on ways to minimise the spillage of imported grains during unloading and transport.

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