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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

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2. Summary

Transportation and handling activities in the course of import of genetically modified (GM) oilseed rape (OSR, *Brassica napus*) have been identified to be the main reason for the unintended occurrence and subsequent establishment of feral GM OSR populations in countries without GM OSR cultivation e.g. Japan and Switzerland. To investigate the presence and frequency of feral OSR plants that descended from seed spillage during transport activities of conventional domestic and imported OSR loads in Austria – a country where import of GM OSR is banned –, feral OSR was sampled for genetic analyses in the present project. The sampling procedure for our sites dispersed throughout Austria considered **transport routes** (railways, roads) as well as **handling and loading sites** for OSR such as railway stations, switchyards, ports, oil mills, and processing facilities. Due to requirements for permissions, involving excessive planning and safety concerns, we did neither include railway and motorway sections nor river course sections. In total, **60 sample sites** were chosen. These included 10 railway stations within Austrian OSR cultivation areas and 10 stations outside OSR cultivation areas; 6 railway stations along the borders to other countries; 2 switchyards; 6 ports (3 with known OSR loading activities); 3 oil mills that import OSR from abroad; 1 processing company; 11 transportation roads within Austrian OSR cultivation areas and 11 roads outside cultivation areas. These sample sites include **predefined hotspots** where OSR seed spillage is expected to occur to a high degree because of handling activities associated with a higher risk for spillage (e.g. oil mills or ports) as well as **randomly selected sample sites** where OSR seed spillage is possible (e.g. railway stations or road sections along transportation routes within Austrian OSR cultivation areas and outside these areas). Feral OSR plants growing along transportation routes outside the OSR cultivation areas are expected to be more likely to have originated from seed spillage of imported OSR than from transport of OSR seeds harvested in Austria.

A total of **2,113 OSR individuals** of feral OSR were sampled on the 60 sites from spring to summer in 2014 and 2015. At all sample sites additional information concerning population size, growing conditions and stage of maturity (blossoms, fruits, seeds) for example was collected. Young leaves were taken from each plant and immediately dried with silica-gel. DNA of the dried leaves was extracted in the lab using a commercial kit (DNeasy 96 Plant Kit). Seed samples from OSR varieties that were grown during the last ten years in Austria were organised from three of four Austrian OSR breeding companies, namely the two main companies Saatzucht Donau GesmbH & CoKG and Raiffeisenware Austria (RWA AG) as well as the KWS Austria Saat GmbH. Because of confidentiality concerns it was not possible to obtain seed samples from the company Pioneer Hi Bred Services GmbH which has an OSR market share in Austria of about 1–3%. Altogether 48 OSR varieties – including eight varieties from other EU countries that were not grown in Austria – and additionally three seed samples from the warehouse in Marchegg, the Oil Mill Fandler and the Oil Mill Raab – were characterised. Although germination trials were repeated, the seeds of five variety seed samples did not germinate at all due to the decreasing seed germination capacity of these old varieties. Eight microsatellite (SSR) primers (Na12-A08, Na12-C06, Na-C08, Na12-C12, Na12-D11, Na12-E01, Na12-E06A, Na10-C01; the first seven have been successfully applied in previous studies: Pascher et al. 2000, 2006, 2010) that amplify ten microsatellite loci have been used in an attempt to identify the origin of the sampled feral plants *via* SSRs, assuming that certain allelic variants should be associated with the organised 48 OSR varieties or at least with variety gene pools. It was shown that several additional *Brassica napus* microsatellite loci would be necessary to explicitly characterize each of the commercial OSR varieties used during the last ten years.

The following results of the present study were obtained:

1. Inquiry and availability of essential transportation data

- Since 2012, amendments based on EU-guidelines led to new classifications of goods. At this time, OSR was incorporated into the category of goods *"other products of vegetable origin" ("Andere Erzeugnisse pflanzlichen Ursprungs"*). Hence, OSR is not recorded separately, which makes traceability impossible.

- It is completely unknown which specific OSR varieties are either imported to Austria or transported through Austria. According to personal communication from managers of warehouses, ports, railway stations, etc., several OSR varieties are exported to Austria as bulk mixtures, but all complying with the quality standards concerning oil content, absence of GM material and low content of erucic acid and glucosinolates. Hence, the identity of origin of a variety ("Herkunftsidentität der Sorten") cannot be obtained and traceability is not possible.

- It was not possible to receive variety-specific data, neither from Statistik Austria nor from the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management (IACS data, German INVEKOS) because of confidentiality concerns. Oil mills and warehouses with few exceptions did not provide such data either.

- At present, **Hungary**, **Serbia** and **Slovakia** are the main OSR exporters to Austria.

- Comparatively detailed data exist for transportation *via* ship. In contrast, **only rough data concerning transport *via* railway and trucks** are available. For instance, the roads that the truck drivers use for seed carriage are not predefined by the oil mills and are therefore unknown. The truck-drivers choose their driving routes on their own.

- Due to weak implementation of the polluter pays principle and incomplete cost calculations concerning external costs in the transport market, the rail transport sector, lost most of its market shares to the road transport sector. This is not in line with political mobility targets and environmental politics, but up to now it is a fact.

- Summarizing, a large information gap could be identified during our inquiry concerning data of OSR varieties and their handling and transportation.

2. Seed spillage and establishment of feral OSR plants in Austria

- Occurrence of feral OSR plants was repeatedly confirmed along relevant transport routes of goods traffic. Especially, the outside curves are sites where many seeds are spilled.

- Feral OSR originates from accidental spillage of viable seeds during transport activities (truck, railway, ship) as well as during loading and handling (e.g. oil mill, switchyard, port). At all of these sites feral OSR is frequently found.

- At **44 sites** of altogether 60 assigned sample sites, feral OSR was registered in 2014 and/or in 2015.

- Establishment of feral OSR populations was found to be significantly more likely in Austrian OSR cultivation areas than in areas where no OSR was grown.

- In several cases feral OSR occurred at these sites in large numbers (e.g. up to 1,500 plants on a road section of 2 kilometres in Upper Austria; more than thousands of individuals were present on the company premises of the oil mill of Bunge).

- **Railway lines** were an ideal habitat for the establishment of feral OSR. Due to the application of herbicides along railway lines, these are expected to be particularly favourable to be colonised by herbicide resistant (HR) OSR.

- Most of the feral OSR plants exhibited high vitality. They blossomed and had already developed viable seeds which are the prerequisites for the persistence of a population over years.

- Most of the OSR populations registered in 2014 were still present at the same sites in 2015, which is an evidence for their persistence.

- Several of more than 20 closely related species in Austria which are known to hybridise with OSR could be registered in the 60 sample sites. Especially *Sinapis arvensis* and *Diplotaxis tenuifolia* were frequently found at the sites throughout Austria.

3. Characterisation of OSR variety, assignment of feral OSR to variety gene pools and genetic diversity

- In total, **2,113 feral OSR plants** were sampled during 2014 and 2015. 1,855 of these were analysed with microsatellites (eight markers) in the present project. Additionally, 217 individuals of 45 out of 48 OSR varieties (i.e., on average 4.8 individuals per variety) were investigated in the same way. Deviations between number of collected samples (and varieties) and the actual number of processed samples were due to technical reasons (e.g. repeated amplification failure, failure to germinate). In contrast to previous studies (PASCHER et al. 2000, 2006, 2010), single OSR varieties could not be unambiguously characterised with the applied SSR markers, although they included the same set of markers. Structure analysis – using DeltaK statistics – resolved the presence of three gene pools. Most varieties were genetically rather uniform, i.e. individuals of the same variety had similar – admixed or not-admixed – genotypic composition. With few exceptions, varieties of the same breeder belonged to the same gene pool, as expected, because usually they can be traced back to the same source variety ("Ursprungssorte").

- During the last years hybrid varieties have gained increasing importance. Currently 76% of all OSR varieties are hybrids and for this reason dominate the Austrian market. Also GM OSR varieties are exclusively hybrids because they are easier to be obtained using that breeding technique. Single genes of the descendants separate following the Mendelian inheritance. According to that, all individuals of the F1 generation are genetically uniform hybrids. Single genes of the F2 generation split into 25% equal to line A (first parent), 50% heterozygous (hybrids) and 25% equal to those of line B (second parent). That means it is possible to have feral plants in our data set which carry specific traits that are genetically closer to their parental source lines.

- Each of the three gene pools identified in the varieties was found in all groups of habitats. That means that no genetic differentiation between sampled habitat groups could be found. Hence, it was also not possible to assign the feral plants to single OSR varieties.

- Analysis of molecular variance calculated with AMOVA showed that > 95% of the total genetic variance was within populations and only up to less than 5% were among habitat types. This was in accordance with the distribution of the three gene pools in all habitat types.

- A comparison of genetic diversity within populations showed that it was highest at oil mills and ports, whereas **border railway stations and transportation roads had the lowest genetic diversity**. Oil mills and ports are those sites where loading and handling of OSR seeds is performed and where, therefore, spillage of imported seeds is expected to be most probable. In contrast, spillage along transportation roads likely traces back to loss of seeds from local transport (i.e. trucks of Austrian farmers) due to inadequate containment of the truck load.

4. Identification of the most sensitive links for seed spillage of imported OSR within the transportation and processing chain

- **Reloading and processing facilities of OSR** (e.g. ports, switchyards, oil mills) are hotspots of spillage of imported seeds.
- **Border railway stations e.g. to Italy** because different voltage is used for running the trains (Austria 15 kV, Italy 3 kV). Transformation is necessary. So the trains are not able to pass the border zone but have to slow down and come to a stop at the border.
- **Railway stations** (e.g. Innsbruck) where **defect trains** are repaired.
- **Omitting of controls at the Austrian borders**: If unloading hatches ("Entleerungsklappen") on the goods wagons are defect or not carefully closed, OSR seeds will be spilled along wide sections of railway lines.
- **Frangible or inadequate containment** of trucks
- **Mode of transportation** of OSR seeds (open loading areas, goods carriage, big bags)
- **Insufficient cleaning measures of loading areas** of trucks and ships as well as goods wagons
- **Insufficient cleaning measures of processing facilities** (oil mills etc.)
- **Applied herbicide management**
- **Wheat imports from Canada** have been identified as potential source for contamination with GM OSR seeds in Switzerland (SCHULZE et al. 2015). At present, it has no relevance for Austria because no wheat is imported from Canada. But that issue should be considered as a possible cause for accidental contamination in future.

If monitoring of GM OSR spillage was performed under financial constraints, it should focus on reloading and handling sites such as ports, processing facilities (oil mills) and switchyards. Relevant contact addresses and sources for necessary information are provided in the present project and may be updated immediately.

Measures to prevent spillage of imported GM OSR seeds and their establishment should concentrate on herbicide application and also on intensification of alternative weed management such as the manual removal of single plants (effective in case of herbicide resistant OSR) or spraying of organic herbicides. Also the used mode of seed packing during transportation (loose transport, big bags, etc.) is an essential factor for limiting seed spillage. More intense controls should be carried out at railway borders in Austria to prevent spillage due to inappropriate transportation facilities.

The Austrian import bans for the genetically modified herbicide tolerant (GMHT) OSR lines Ms8, Rf3 and Ms8xRf3 (BGBl. Nr. II 317/2012) and GT73 (BGBl. Nr. II 318/2012) were in force until 30th of November 2015. Meanwhile, the bans have been prolonged up to 2018. The recent scientific results of the present study underline the Austrian justification based on the reviews of PASCHER (2011, 2012) for the import ban of the GMHT OSR lines Ms8xRf3 and GT73 with new evidence. Hence, from a scientific point of view the import bans of these lines are recommended to be prolonged.

3. Zusammenfassung

Transportaktivitäten und Hantieren im Zuge des Imports von gentechnisch verändertem (GV) Raps (*Brassica napus*) wurden als Hauptursachen für das unbeabsichtigte Vorkommen und die anschließende Etablierung von verwildertem GV Raps in Ländern identifiziert, in denen kein GV Raps angebaut wird, wie etwa in Japan oder in der Schweiz. In Österreich sind aktuell für den Import von GV Raps Verbotsverordnungen in Kraft. Um das Vorkommen und die Häufigkeit von verwilderten Rapspflanzen zu untersuchen, die aus Samenverstreuungen während Transportaktivitäten von konventionellen heimischen und importierten Rapsladungen in Österreich hervorgegangen sind, wurde im Rahmen der Studie verwilderter Raps für genetische Analysen gesammelt. Das Design der Probennahme auf den ausgewählten Untersuchungsstandorte, die über ganz Österreich verstreut waren, berücksichtigte sowohl **Transportrouten** (Bahnstrecken, Straßen) als auch **Hantierungs- und Verladestellen** von Raps wie etwa Bahnhöfe, Grenz- und Frachtenbahnhöfe, Hafenanlagen, Ölmühlen und Verarbeitungsbetriebe. Aufgrund von erforderlichen Genehmigungen, aufwändiger Planung und Sicherheitsgründen wurde auf die Beprobung von Streckenabschnitten der Bahnlinien und der Autobahn verzichtet. Auch zufällig gewählte Flussabschnitte wurden nicht in die Beprobung miteinbezogen. Es wurden insgesamt **60 Untersuchungsflächen** ausgewählt. Diese inkludierten 10 Bahnhöfe innerhalb der österreichischen Rapsanbaugebiete; 10 Bahnhöfe, die außerhalb dieser situiert waren; 6 Grenzbahnhöfe; 2 Frachtenbahnhöfe; 6 Hafenanlagen, von denen bei 3 bekannt war, dass sie Raps verladen; 3 Ölmühlen, die Raps aus dem Ausland importieren; 1 Verarbeitungsbetrieb; 11 Transportstraßen innerhalb von Rapsanbaugebieten und 11 Straßen außerhalb dieser Anbaugebiete. Die Untersuchungsstandorte beinhalteten einerseits **vorgegebene Hotspots**, in denen die Verstreuung von Rapssamen aufgrund der durchgeführten Handhabung von Raps mit großer Wahrscheinlichkeit zu erwarten ist und die dadurch ein erhöhtes Risiko für Samenverluste aufweisen (z.B. Ölmühlen und Häfen), andererseits **zufällig gewählte Untersuchungsstandorte**, auf denen Rapssamenverluste prinzipiell möglich sind (z.B. Bahnhöfe oder Straßenabschnitte entlang von Transportrouten innerhalb und außerhalb von Rapsanbaugebieten). Es ist generell anzunehmen, dass Ruderalraps, der entlang von Transportrouten außerhalb der Rapsanbaugebiete wächst, mit größerer Wahrscheinlichkeit von Samenverlusten von importiertem Raps stammt als von lokalen Transporten im Zuge von landwirtschaftlichen Tätigkeiten der österreichischen LandwirtInnen.

Von insgesamt **2,113 gesammelten verwilderten Rapsindividuen** auf den 60 Untersuchungsflächen wurde Blattmaterial in den Jahren 2014 und 2015 von Frühling bis Sommer gesammelt. Auf allen Untersuchungsstandorten wurden zusätzliche Informationen beispielsweise über Populationsgrößen, Standortbedingungen und das Reifestadium (Blüten, Früchte, Samen) der Pflanzen festgehalten. Die gepflückten Stängelblätter wurden sofort mit Silika-Gel getrocknet. Im Labor wurde die DNA der getrockneten Blätter mit dem kommerziellen Kit (DNeasy 96 Plant Kit) extrahiert. Sortenmuster der konventionellen Rapssorten, die während der letzten zehn Jahre in Österreich für den Anbau zur Verfügung standen, wurden von drei der vier österreichischen Rapszüchter organisiert, von den zwei Hauptunternehmen der Saatzucht Donau GesmbH & CoKG und der Raiffeisenware Austria (RWA AG), und von der kleineren Züchtungsfirma KWS Austria Saat GmbH. Aus Vertraulichkeitsgründen war es leider nicht möglich, Sortenmuster der Firma Pioneer Hi Bred Services GmbH für unsere Studie zu erhalten, deren Rapssorten einen Marktanteil von etwa 1-3% abdecken. Insgesamt wurden 48 Rapssorten – von diesen stammten 8 aus anderen EU Ländern, die nicht in Österreich angebaut wurden – und zusätzlich drei Sortenmuster von Samenmischungen, je eines vom Lagerhaus in Marchegg, der Ölmühle Fandler und der Ölmühle Raab, charakterisiert. Trotz der Wiederholung der Keimungsversuche wurde bei den Samen von fünf Sortenmustern kein Keimungserfolg erzielt, was voraussichtlich auf die abnehmende Keimungsfähigkeit älterer Samen von früher angebauten Sorten zurückzuführen ist. Acht Mikrosatelliten (SSR) Primer (Na12-A08, Na12-C06, Na-C08, Na12-C12, Na12-D11, Na12-E01, Na12-E06A, Na10-C01; die ersten sieben wurden erfolgreich in vorangegangenen Studien angewendet: PASCHER et al. 2000, 2006, 2010), die zehn Mikrosatelliten-

Loci amplifizieren, wurden verwendet, um die Herkunft der gesammelten verwilderten Rapspflanzen zu identifizieren. Es wurde angenommen, dass bestimmte Allel-Varianten den 48 organisierten Rapssorten oder zumindest gemeinsamen Sorten-Genpools zugeordnet werden könnten. Es stellte sich nach den Analysen heraus, dass mehrere zusätzliche *Brassica napus*-Mikrosatelliten notwendig wären, um jede der konventionellen Rapssorten, die in den letzten zehn Jahren in Österreich angebaut wurde, eindeutig charakterisieren zu können.

Die folgenden Ergebnisse konnten in der vorliegenden Studie gewonnen werden:

1. Recherche und Verfügbarkeit von wichtigen Transportdaten

- Seit dem Jahr 2012 haben Abänderungsanträge, die auf EU-Richtlinien beruhen, eine neue Klassifikation von Gütern bewirkt. Ab diesem Jahr wurde Raps in die Kategorie von Gütern *"Andere Erzeugnisse pflanzlichen Ursprungs"* inkorporiert. Aus diesem Grund wird Raps nicht mehr separat aufgelistet, was dessen Rückverfolgbarkeit unmöglich macht.

- Es ist völlig unbekannt, welche konkreten Rapssorten nach Österreich importiert oder durch Österreich transportiert werden.

- Persönlichen Gesprächen mit den Leitern von Lagerhäusern, Hafenanlagen, Bahnhöfen, etc. zufolge werden sämtliche Rapssorten von Exportländern nach Österreich als Sortengemische transportiert, die alle bestimmte Qualitätsstandards wie Ölgehalt, niedriger Gehalt an Erucasäure und Glukosinolaten sowie Gentechnikfreiheit erfüllen. Folglich sind die Herkunftsidentität sowie eine Rückverfolgbarkeit der Sorten nicht gegeben.

- Zudem war es aus Vertraulichkeitsgründen nicht möglich, weder von der Statistik Austria noch vom Bundesministerium für ein lebenswertes Österreich, dem BMLFUW (INVEKOS-Daten), sortenspezifische Daten zu erhalten.

- Derzeit sind die Länder **Ungarn, Serbien und Slowakei** die Hauptexporteure von Raps nach Österreich.

- Vergleichsweise detaillierte Daten liegen für den Transport von Raps per Schiff vor. Im Gegensatz dazu sind nur **sehr ungenaue Daten über den Transport mit der Bahn oder mit Lastwägen** verfügbar. So sind beispielsweise die Straßenrouten, auf denen die Lastwagenfahrer die Rapssamen transportieren, von den Ölmühlen nicht vorgegeben und aus diesem Grund nicht nachvollziehbar. Die Lastwagenfahrer wählen ihre Fahrtrouten selbständig.

- Aufgrund von massiven Wettbewerbsverzerrungen im Transportsektor - unvollständige Anwendung des Verursacherprinzips und unvollständige Kostenrechnung v.a. hinsichtlich externer Kosten („cost-by-cause") - kann der Straßengütertransport zu wesentlich billigeren Preisen erfolgen. Dies hat, entgegen aller verkehrspolitischer Ziele, dazu geführt, dass der Gütertransport auf der Schiene auch beim Raps Marktanteile an den LKW verloren hat.

- Resümierend kann gesagt werden, dass während unserer Recherche eine große Informationslücke betreffend der Herkunftsidentität von einzelnen Rapssorten sowie der Hantierung und des Transports von Rapssamen identifiziert werden konnte.

2. Samenverluste und Etablierung von verwilderten Rapspflanzen in Österreich

- Das Vorkommen von Ruderalraps entlang von relevanten Transportrouten des Güterverkehrs wurde wiederholt bestätigt. Vor allem die Außenkurven stellen Bereiche dar, in denen es zu besonders großen Samenverlusten kommen kann.

- Verwilderter Raps geht auf zufällige Samenverstreuung von lebensfähigen Samen während Transportaktivitäten (Lastwägen, Bahnlinien, Schiffe) sowie Verladungs- und Hantierungs-tätigkeiten (z.B. Ölmühlen, Grenz- und Frachtenbahnhöfe, Hafenanlagen) zurück. Auf allen diesen Standorten konnte Ruderalraps regelmäßig gefunden werden.

- Auf **44** der insgesamt 60 festgelegten Untersuchungsstandorte konnte 2014 und/oder 2015 Ruderalraps registriert werden.

- Es hat sich herausgestellt, dass die Etablierung von verwilderten Rapspopulationen signifikant wahrscheinlicher in den österreichischen Rapsanbaugebieten ist als außerhalb von diesen.

- In mehreren Fällen trat verwilderter Raps auf den Untersuchungsstandorten in großer Zahl auf, z.B. mehr als 1.500 Individuen auf einem Straßenabschnitt von 2 km; Tausende Rapspflanzen auf dem Gelände der Ölmühle Bunge.

- **Bahngleiskörper** stellten sich als ein idealer Lebensraum für die Etablierung von verwildertem Raps heraus und wären es aufgrund des angewendeten Herbizid-Managements entlang der Bahngleiskörper auch im Besonderen für herbizidresistenten (HR) Raps.

- Die meisten der verwilderten Rapspflanzen wiesen eine hohe Vitalität auf. Sie blühten und hatten bereits lebensfähige Samen ausgebildet, was die Voraussetzung für die Persistenz der Population über Jahre ist.

- Die 2014 registrierten Rapspopulationen konnten in fast allen Fällen am selben Untersuchungsstandort auch 2015, das heißt, in zwei aufeinanderfolgenden Jahren, nachgewiesen werden, was deren Persistenz bestätigt.

- **Einige von mehr als 20 nahe verwandten Arten** in Österreich, die mit Raps hybridisieren können, konnten auf den 60 Untersuchungsstandorten registriert werden. Besonders *Sinapis arvensis* und *Diplotaxis tenuifolia* waren auf den Standorten in ganz Österreich sehr häufig vorhanden.

3. Charakterisierung von Rapssorten, Zuordnung von Ruderalraps zu Sorten-Genpools und genetische Diversität

- Es wurden insgesamt **2.113 verwilderte Ruderalpflanzen** 2014 und 2015 beprobt. 1.855 von diesen konnten im vorliegenden Projekt mit Mikrosatelliten - acht Marker - analysiert werden. Zusätzlich wurden 217 Individuen von 45 der insgesamt 48 organisierten konventionellen Rapssorten (im Durchschnitt 4,8 Individuen pro Sorte) in derselben Weise charakterisiert. Abweichungen in den Zahlen der gesammelten Proben zusammen mit den Sorten und der tatsächlich analysierten Proben gehen auf technische Schwierigkeiten wie etwa wiederholte Amplifikationsausfälle oder Keimungsprobleme der Samen zurück. Im Gegensatz zu vorangegangenen Studien der Autoren (Pascher et al. 2000, 2006, 2010) war es in dieser Studie nicht möglich, einzelne Rapssorten mit den angewendeten SSR-Markern eindeutig zu charakterisieren, obwohl der gleiche Satz an Markern verwendet wurde. Structure Analysis unter

Verwendung von DeltaK Statistik zeigte das Vorhandensein von drei Genpools. Die meisten Sorten waren genetisch überwiegend einheitlich, das heißt, die Individuen der gleichen Sorte wiesen ähnliche genotypische Komposition auf. Mit wenigen Ausreißern gehörten, wie zu erwarten, die Sorten des gleichen Züchters zum gleichen Genpool, da sie normaler Weise auf die gleiche Ursprungssorte zurückgehen.

- Im Laufe der letzten Jahre gewannen Hybridsorten zunehmend an Bedeutung. Aktuell sind etwa 76% von allen konventionellen Rapssorten Hybride und dominieren aus diesem Grund auch den österreichischen Markt. Auch GV Rapssorten sind ausschließlich Hybride, da sie mit dieser Züchtungstechnik erfolgreicher hergestellt werden können. Einzelne Gene der Nachkommen teilen sich in der F2 Generation den Mendelschen Regeln folgend auf. Demzufolge sind alle Individuen der F1 Generation genetisch uniforme Hybride. Einzelne Gene der F2 Generation teilen sind in 25%, die ident zur Elternlinie A sind, 50% Heterozygote (Hybride) und 25%, die ident zur Elternlinie B sind, auf. Daraus kann gefolgert werden, dass es möglich ist, dass in unserem Datensatz auch verwilderte Rapspflanzen vorhanden sind, die spezifische Eigenschaften tragen, die genetisch näher zu den elterlichen Ursprungssorten sind.

- Das Vorhandensein von jedem der drei Genpools, die in den analysierten Sorten identifiziert werden konnten, wurde in allen Lebensraum- (Habitat) Gruppen bestätigt. Das bedeutet, dass keine genetische Differenzierung zwischen den beprobten Habitattypen gefunden werden konnte. So war es auch nicht möglich, verwilderte Rapspflanzen einzelnen Rapssorten zuzuordnen.

- Die Analyse der Molekularen Varianz, die mit dem Programm AMOVA berechnet wurde, zeigte, dass **> 95% der gesamten genetische Varianz innerhalb der Populationen** vorkam und nur weniger als 5% innerhalb der Habitattypen. Dieses Ergebnis stimmt mit dem Vorkommen der drei Genpools in allen Habitatgruppen überein.

- Ein Vergleich der genetischen Diversität zwischen Populationen zeigte, dass diese am höchsten in Ölmühlen und Hafenanlagen war, während **Grenzbahnhöfe und Transportstraßen die niedrigste genetische Diversität** aufwiesen. Ölmühlen und Häfen sind gerade diejenigen Stellen, an denen Verladung und Hantierung mit Rapssamen stattfinden und wo aus diesem Grund auch mit relativ hoher Wahrscheinlichkeit Verluste von importierten Rapssamen zu erwarten sind. Im Gegensatz dazu geht Samenverstreuung entlang von Transportwegen zum Großteil voraussichtlich auf mangelhafte Abdeckung des Ladeguts auf Ladeflächen von Landwirtschaftsfahrzeugen während lokaler Transporttätigkeit, das heißt also, auf Samenverluste verursacht durch die österreichischen LandwirtInnen selbst zurück.

4. Identifizierung von sensiblen Gliedern in der Transport- und Verarbeitungskette bezüglich Samenverlust von importiertem Raps

- **Verlade- und Verarbeitungseinrichtungen von Raps** (z.B. Häfen, Frachtenbahnhöfe, Ölmühlen) sind Hotspots für Samenverluste von importierten Samen.
- **Grenzbahnhöfe z.B. nach Italien**, da verschiedene Spannungen für die Betriebsführung von Zügen verwendet werden (Österreich 15 kV, Italien 3 kV), was eine Transformation notwendig macht. Die Züge können die Grenze nicht passieren, sondern müssen abbremsen und zum Stillstand kommen.
- **Bahnhöfe** (z.B. Innsbruck), in denen **defekte Züge und Wagons** repariert werden.
- **Kontrollaufgabe an den österreichischen Grenzen:** Wenn Entleerungsklappen der Güterwagons defekt oder nicht sorgfältig geschlossen sind, können Rapssamen über weite Strecken der Bahnlinie verloren gehen.
- **Spröde Dichtungen oder unzureichende Abdeckung** von Lastwägen.

- **Aufbewahrung der Rapssamen während des Transports** (offene Ladeflächen, Güterbeförderung, Big Bags).
- **Unzureichende Reinigungsmaßnahmen von Ladeflächen** von Lastwägen und Schiffen als auch von Zugwagons.
- **Unzureichende Reinigungsmaßnahmen von Verarbeitungseinrichtungen** (Ölmühlen, etc.).
- **Angewendetes Herbizidmanagement**
- **Kanadische Weizenimporte wurden als potentielle Quelle für Kontaminationen mit GV Rapssamen in der Schweiz identifiziert** (SCHULZE et al. 2015). Derzeit hat diese Thematik keine Relevanz für Österreich, da kein Raps aus Kanada importiert wird. Allerdings sollte diese mögliche Ursache von zufälligen Kontaminationen mit GV Raps in der Zukunft nicht außer Acht gelassen werden.

Wenn man ein Monitoring von Importverlusten von GV Rapssamen unter finanziellen Beschränkungen durchführen müsste, sollte ein Schwergewicht der Beprobung auf Verlade- und Hantierungsstellen wie etwa Hafenanlagen, Verarbeitungseinrichtungen (Ölmühlen) und Frachtenbahnhöfe gelegt werden. Relevante Kontaktadressen und Quellen für wichtige Informationen werden im vorliegenden Projekt bereitgestellt und können bei Bedarf sofort aktualisiert werden.

Maßnahmen, um Samenverluste von importiertem GV Raps und dessen Etablierung zu verhindern, sollten auf dem Einsatz von Herbiziden, jedoch auch auf einer intensiveren Anwendung von alternativen Unkrautbekämpfungsstrategien, wie etwa dem händischen Entfernen von Einzelpflanzen (v.a. wirksam bei herbizidresistentem Raps) oder ökologischer Beikrautkontrolle, fokussieren. Auch die verwendete Samenverpackungsform während des Transports (offener Samentransport, Big Bags, etc.) stellt einen wesentlichen Faktor für die Begrenzung von Samenverlusten dar. Kontrollen an den österreichischen Grenzübergängen der Bahn sollten intensiviert werden, um Samenverstreuung aufgrund defekter Transporteinrichtung entgegenwirken zu können.

Die österreichischen Importverbote für die gentechnisch veränderten herbizidresistenten Rapslinien Ms8, Rf3 und Ms8xRf3 (BGBl. Nr. II 317/2012) und GT73 (BGBl. Nr. II 318/2012) waren bis zum 30. November 2015 in Kraft und wurden in der Zwischenzeit bis 2018 verlängert. Die vorliegende Studie untermauert die österreichische Begründung für das Importverbot der GVHT Rapslinien Ms8xRf3 und GT73, die auf den Gutachten von PASCHER (2011, 2012) basiert, mit neuen wissenschaftlichen Ergebnissen. Demzufolge wird aus wissenschaftlicher Sicht empfohlen, die Importverbote für die genannten Rapslinien weiterhin aufrecht zu erhalten.

4. Introduction

Herbicide resistant (HR) genetically modified (GM) oilseed rape (OSR, *Brassica napus*) is cultivated worldwide on large scale in Canada (>90% of OSR cultivation estimated), USA, Chile, and Australia (James 2014). In the European Union cultivation of GM OSR is not authorised at present. However, several OSR lines such as HR GT73 and MS8, RF3, and MS8xRF3 are admitted for import, processing and feed use. In Austria, an import ban for the listed GM OSR lines was in force until November, 30th 2015 and is now prolonged until 2018. The reports of PASCHER (2011, 2012) which are based on literature research verify and underpin repeatedly the ecological concern of the import of GM OSR under Austrian conditions.

Based on scientific evidence, transportation and loading in the course of import activities of GM OSR in countries without GM OSR cultivation are a main pathway for the unintended introduction into the environment and subsequent establishment of feral GM OSR populations outside the cultivation areas of GM OSR (PASCHER 2012). Because of small seed size like pinheads OSR seeds are regularly spilled accidentally during transport and handling activities. Several scientific papers verify that OSR is able to become feral in Europe - which is the area of origin of that crop - supported by the prevailing climatic conditions here. “To become feral” means that the crop OSR is able to grow and establish outside cultivation areas by itself. The plants are no more reliant upon human care and are able to reproduce and persist as population for several years without human support. They frequently occur along transport routes where the seeds were spilled. Research concerning occurrence and persistence of feral OSR populations in EU countries has already been performed in France (PESSEL et al. 2001; PIVARD et al. 2007, 2008; GARNIER et al. 2008), in Germany (DIETZ-PFEILSTETTER et al. 2006; MENZEL 2006; ELLING et al. 2009; MIDDELHOFF et al. 2009), in the Netherlands (TAMIS & DE JONG 2010), in Great Britain (CRAWLEY & BROWN 2004; SQUIRE et al. 2010) as well as in Austria (PASCHER et al. 2000, 2006, 2010). In Switzerland – a country comparable to Austria, both cultivation and import are prohibited – feral GM Glyphosat resistant OSR (GT73) was identified on four of altogether 79 sample sites (SCHOENENBERGER & D’ANDREA 2012). The sites of GM OSR findings were railway stations and ports on the Swiss borders to France and Italy. In the study the occurrence of GM OSR was explained by spillage of contaminated OSR seed from freight trains. GT73 is allowed to be cultivated neither in Switzerland nor in the European Union.

Spillage of GM OSR seeds along transportation routes was also confirmed in Japan (SAJI et al. 2005; KAWATA et al. 2009; NISHIZAWA et al. 2009, 2010; MIZUGUTI et al. 2011), the United States (SAGERS et al. 2010) as well as in Canada (YOSHIMURA et al. 2006; KNISPEL et al. 2008; BECKIE & WARWICK 2010; KNISPEL & MCLACHLAN 2010). The mentioned studies are reviewed in detail in the reports of PASCHER (2011, 2012).

The intention of the present Austrian research project was to document and assess the middle-term to long-term probability of spillage, establishment and distribution of imported viable OSR seeds based on field data collected along transportation routes as well as loading and handling sites. Therefore, fieldwork was repeatedly carried out in spring and summer 2014 and 2015. In accordance with the commission of the Austrian Federal Ministry of Health, the analyses should be based on conventional OSR varieties. The collected feral OSR plants were intended to be genetically analysed by the use of microsatellites. After genetic analyses, they should be associated with the variety gene pools grown in Austria and with the pools which were imported to Austria from other countries during the last years as far as possible. Sites of OSR registration should then be related to the OSR transport networks and the Austrian OSR cultivation areas to the extent as the available data base would allow it.

5. Cultivation of oilseed rape in Austria

Up to date cultivation data of OSR in Austria and total cultivation area were requested from the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management. In this context, IACS data (Integrated Administration and Control System, the German synonym: INVEKOS - Integriertes Verwaltungs- und Kontrollsystem, http://ec.europa.eu/agriculture/direct-support/iacs/index_de.htm) of the present OSR cultivation in Austria were received referring to single parcels ("Feldschläge"). Based on these data, an OSR cultivation map for these municipalities which grew OSR in the year 2012 was created (Figure 1). Figure 2 shows the cultivation proportion of winter OSR in these Austrian municipalities. Figure 3 indicates the cultivation proportion of summer OSR which is cultivated less frequently in Austria. Moreover, cultivation of turnip (*Brassica rapa*) – a closely related oil producing plant which is usually grown in higher altitudes in comparison with OSR – is shown in Figure 4. We also requested information about trade routes of imported OSR, loading and handling sites and ports along the rivers Danube, Enns and Inn over which OSR is traded in. However, these data were not available from this information source. In addition, the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management could not provide any information about the relative proportions of the major OSR varieties grown in Austria during the last years. Additionally, OSR cultivation data for the years 2013 to 2015 were not available from the Ministry in autumn 2015 in a computable form. Hence, it was not possible to update Figures 1 and 2 (Austrian OSR cultivation) in the present report. In the following EUROSTAT table (Table 1), OSR harvest amounts are listed for several EU Member States which grew OSR in the years 2007 to 2012. Fluctuations concerning amounts of grain harvested in different years are common for each country, depending among others on current OSR prices for farmers and growing conditions.

Table 1: Oilseed rape and turnip: harvest amounts in EU Member States in the years 2007-2012 in 1,000 t. Source: EUROSTAT; Union zur Förderung von Öl- und Proteinpflanzen E.V.
http://www.ufop.de/agrar-info/agrar-statistik/

	2007	2008	2009	2010	2011+	2012+
Germany	5,321	5,155	6,307	5,698	3,870	4,819
France	4,684	4,719	5,584	4,816	5,369	5,462
Italy	15	28	51	51	44	49
Netherlands	12	10	12	12	7	9
Belgium / Luxembourg	59	49	60	62	68	67
United Kingdom	2,108	1,973	1,912	2,230	2,778	2,564
Ireland	32	23	22	25	24	28
Denmark	596	629	637	580	508	474
Spain	33	21	35	36	62	52
Austria	145	175	171	171	180	149
Finland	114	89	140	179	115	74
Sweden	222	259	299	279	250	283
EU-15	13,341	13,130	15,230	14,139	13,280	14,036
Estonia	133	111	136	130	124	167
Latvia	212	205	209	225	220	274
Lithuania	312	330	416	415	484	628
Poland	2,130	2,106	2,497	2,078	1,869	1,883
Slovakia	321	424	387	323	332	219
Slovenia	15	11	10	16	14	17
Czech Republic	1,032	1,049	1,128	1,042	1,046	1,127
Hungary	496	655	579	560	527	401
EU-25	18,304	18,351	21,008	19,343	17,896	18,752
Romania	93	231	236	545	732	192
Bulgaria	362	673	570	924	520	386
EU-27	18,759	19,255	21,814	20,812	19,148	19,230

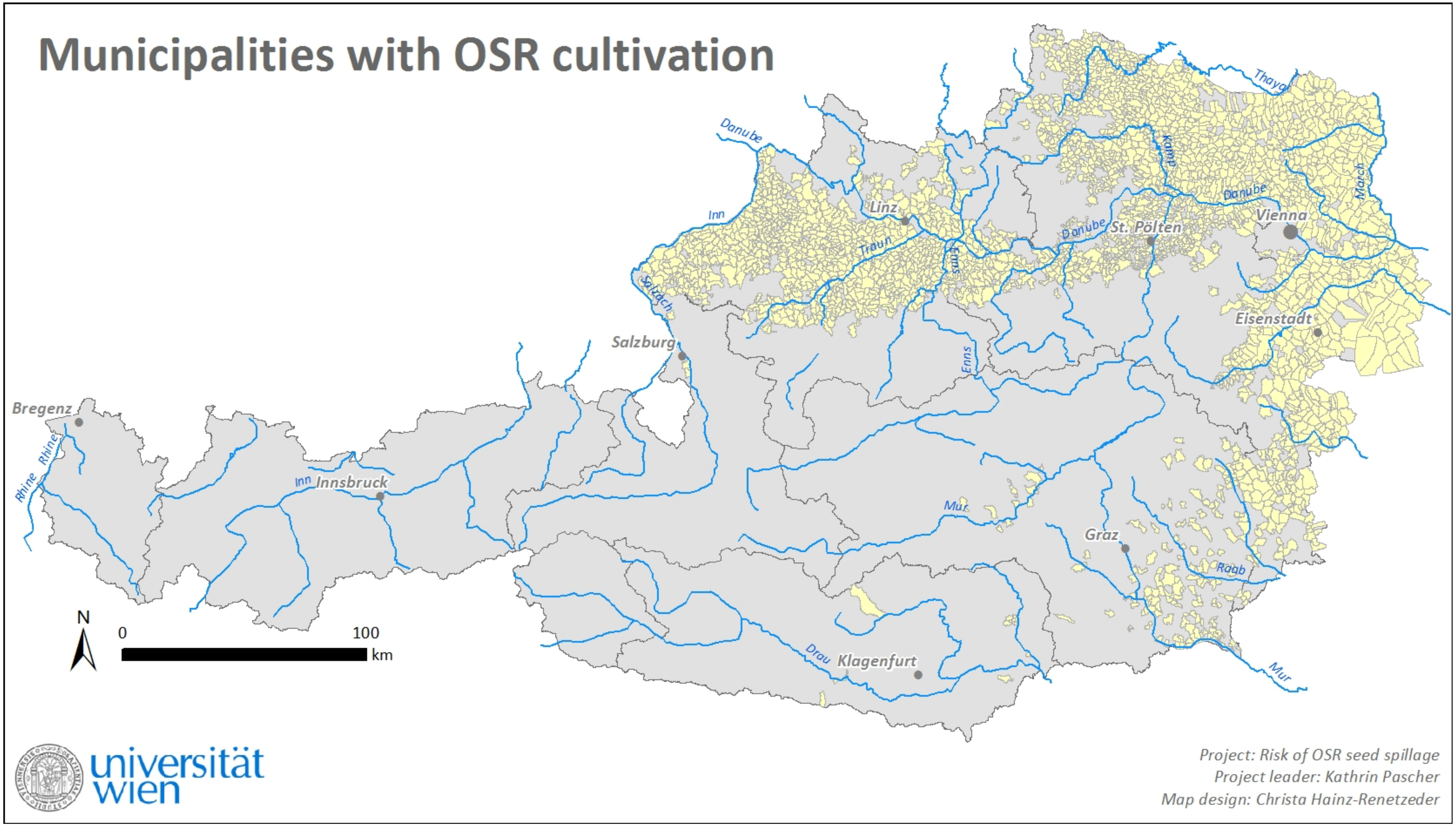


Figure 1: OSR (oilseed rape) cultivation in Austrian municipalities in 2012.

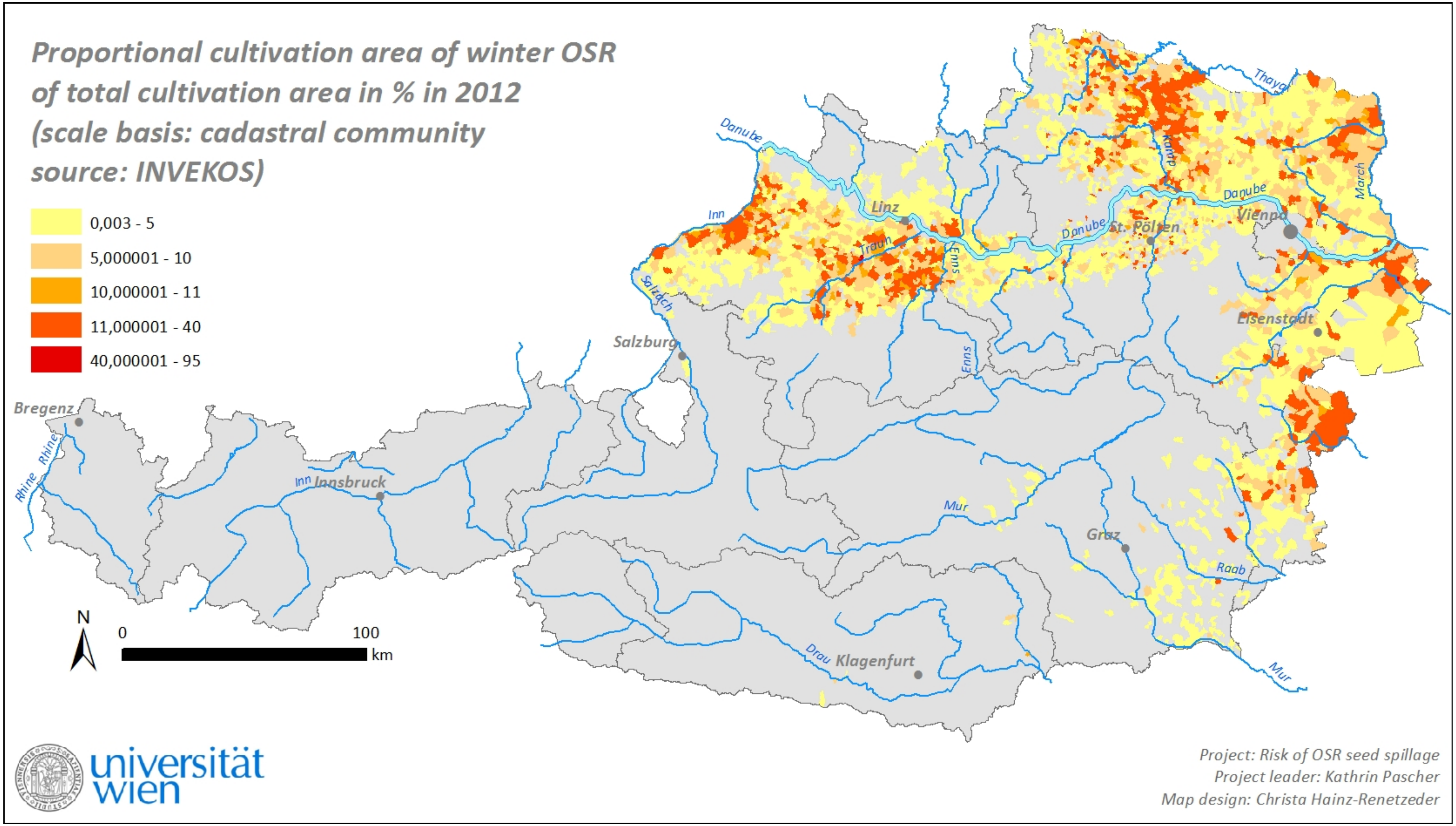


Figure 2: Austrian municipalities with different cultivation extent of winter OSR in % in 2012.

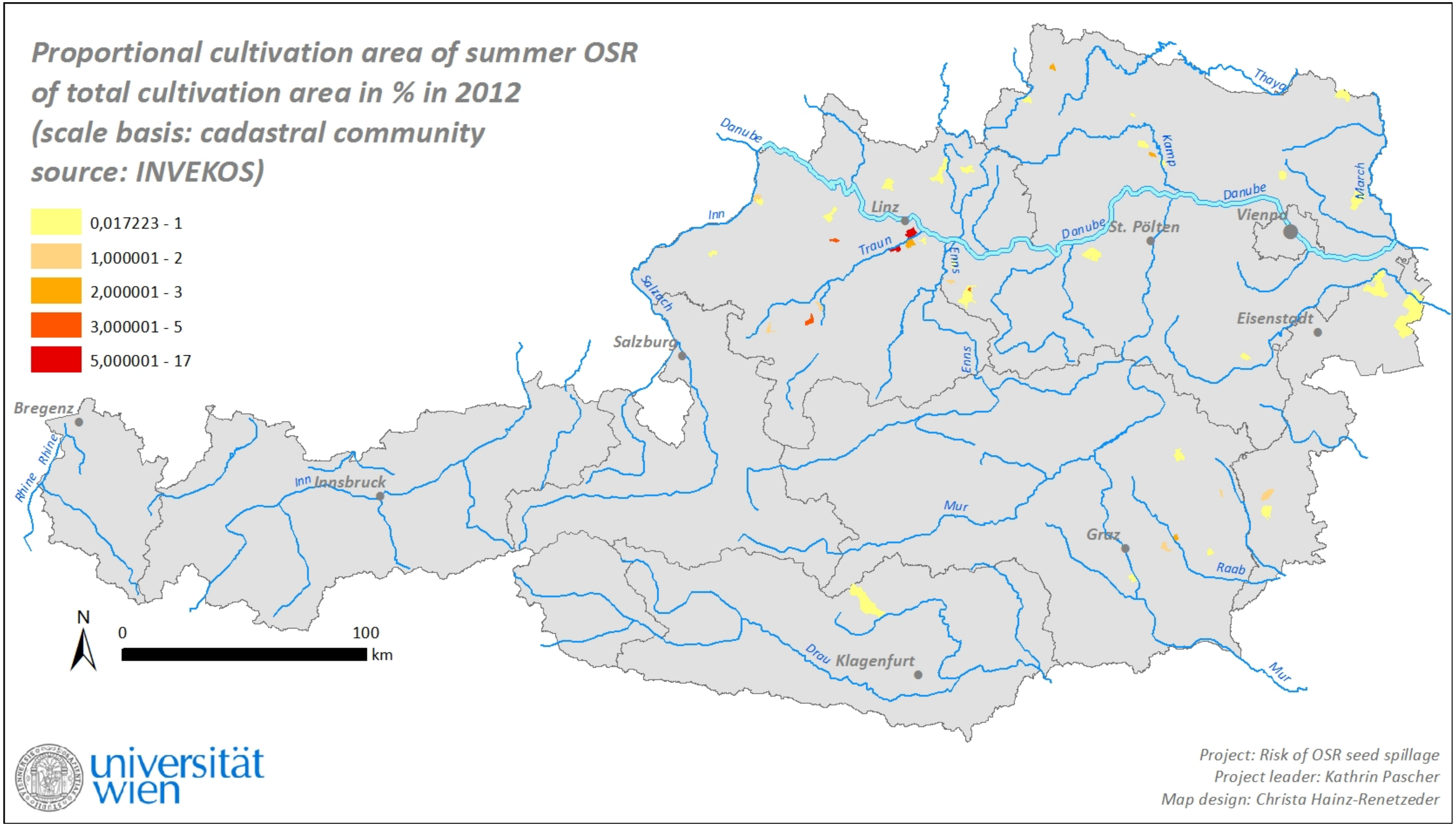


Figure 3: Austrian cultivation area of summer OSR (*Brassica napus*) in % in 2012.

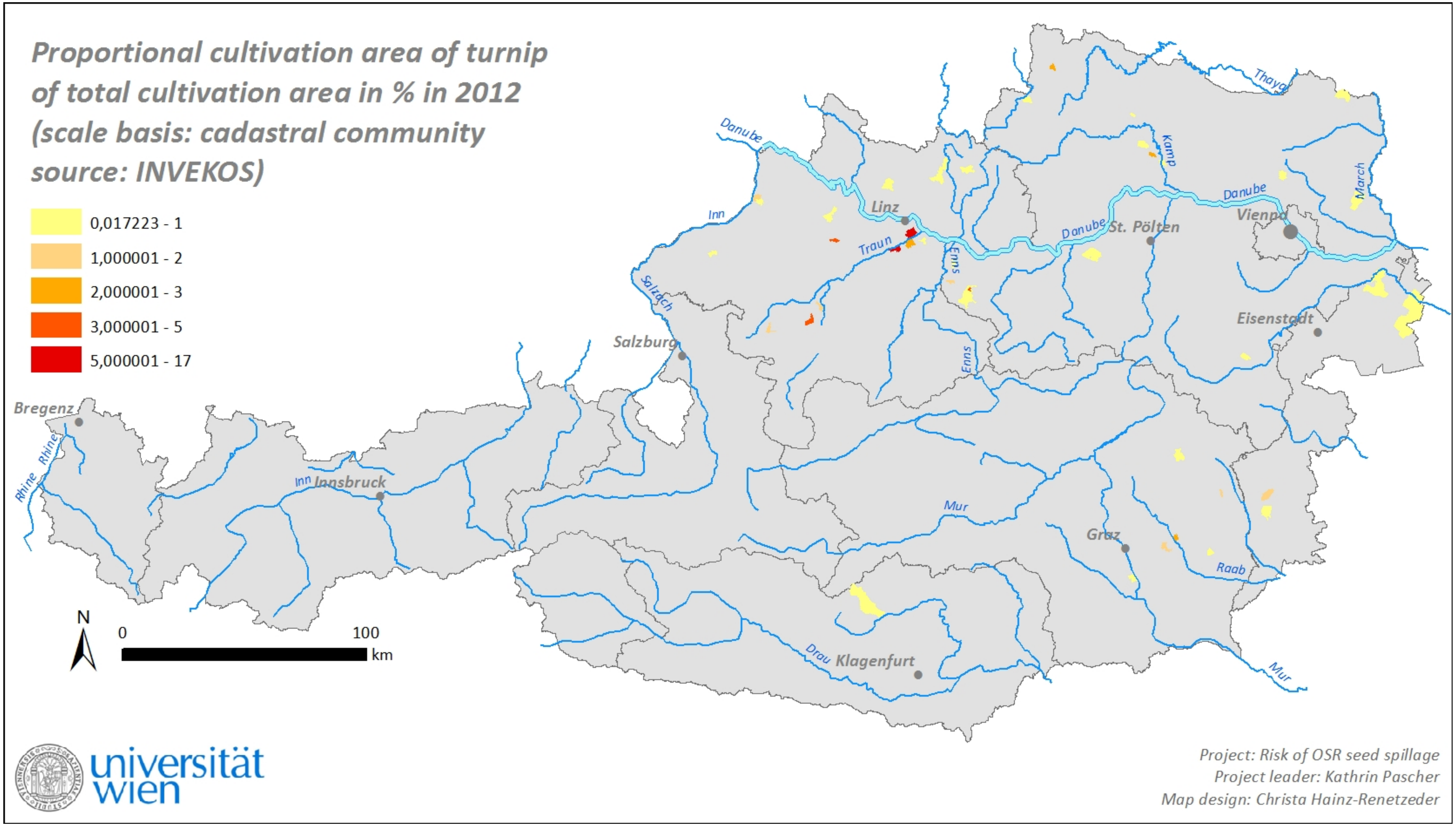


Figure 4: Austrian cultivation area of turnip (*Brassica rapa*) in % in 2012.

6. Inquiry about OSR import in Austria

6.1. Import and transport activities of OSR seeds

In principle, data concerning transportation activities of OSR seeds to and within Austria are difficult to obtain, moreover, some detailed data were unavailable for our project. The most relevant information source during our inquiry proved to be **Statistik Austria** (http://www.statistik.at/) which was able to deliver detailed information concerning import and transportation.

From the Division of Foreign Trade information concerning imported OSR could be obtained. It was not possible to get information about specific OSR varieties which were imported to Austria. Content of oil, low content of erucic acid and glucosinolates, and absence of GM material are the quality standards imported OSR has to comply with. That means it is completely unknown which specific OSR varieties are imported to Austria. According to personal communication with managers from warehouses, ports, railway stations, etc., several OSR varieties are transported to Austria from exporting countries as bulk mixtures, but all complying with the listed quality standards. That means, because of the unavailability of appropriate information it is not possible to reconstruct the identity of origin ("Herkunftsidentität") of OSR varieties in Austria.

The Division of Transport ("Verkehr") provided general data of transport possibilities of imported goods to Austria, but the route chosen by the truck drivers is not recorded at any point. We received detailed data such as imported amounts from respective exporting countries. Detailed data concerning shipping were available. However, only rough data were available for transport *via* railways and roads.

6.1.1. Import of oilseed rape (OSR)

The supply balance sheet for OSR clearly shows the necessity for import of OSR seeds to Austria in order to be able to supply the Austrian market with the oleaginous fruit (Figure 5).

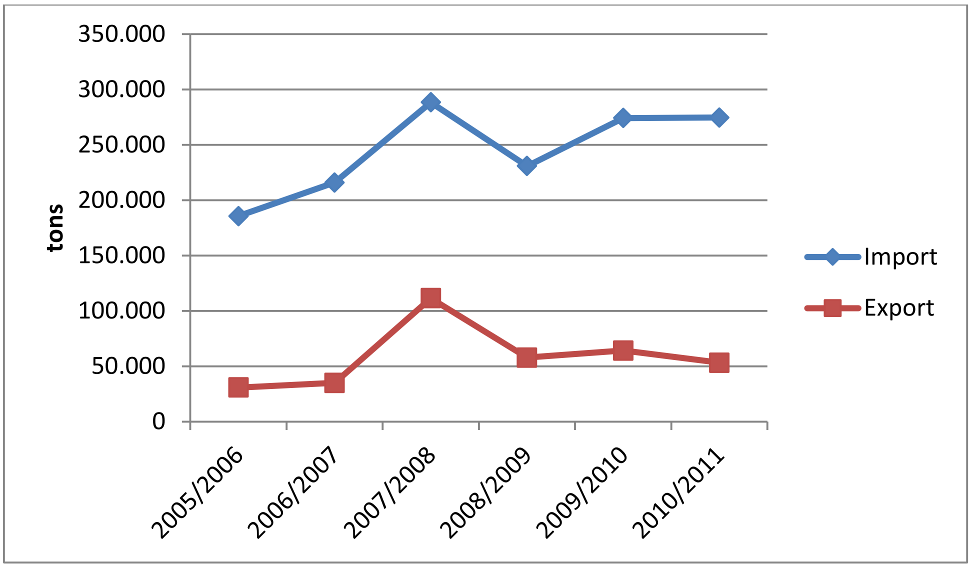


Figure 5: Amount of imported and exported OSR in tons from the year 2005 onwards. Note that a period, instead of a comma, is used as thousands separator according to usage in German language.
Source: Statistik Austria.

Statistik Austria provides very detailed data on OSR importing countries and differentiates between OSR fractions for use as seeds, OSR with different content of erucic acid, OSR with <2%, and OSR with ≥2%. In principle, most of the imported OSR seed originates from European countries. Small amounts are imported from Chile and New Zealand (Figure 6, Figure 7, Figure 8; compare REINER 2006). Especially Hungary, Serbia, and Slovakia exported large amounts of OSR to Austria in the preceding years (Table 2). In total, more than 200,000 tons OSR were transported to Austria in each of the listed years (Table 3). For 2015, data were only available from January to March. Therefore, imported OSR amounts are only registered for that period.

Table 2: Tons of total OSR import to Austria for the years 2012 to 2015, listed by importing countries.

	Total 2012	Total 2013	Total 2014	Total 2015 (Jan-Mar)
Belgium	1	1	1	0
Canada	0	0.15	0.18	0
Chile	0	1	0	0
Croatia	95	359	429	0
Czech Republic	7,616	9,998	1,099	2,284
France	261	330	257	0
Germany	4,134	4,630	3,452	911
Hungary	172,912	119,993	125,139	21,077
Italy	205	0	1,037	250
Moldova	0	69	25	0
Netherlands	23	80	101	57
New Zealand	0	0.06	0	0
Poland	22	62	0	0
Romania	20	0	36	621
Russia	0	10	0	0
Serbia	0	3,642	104,223	0
Slovakia	19,943	109,962	3,913	25,438
Slovenia	3,991	1,763	223	0
Switzerland	589	177	915	199
Ukraine	0	280	0	305
United Kingdom	28	0	0	0

Table 3: OSR import to Austria in tons in the years 2012 until 2015.

Quality and application of imported OSR grain	2012	2013	2014	Jan-Mar 2015
Seeds of oilseed rape with erucic acid < 2%, for sowing	1,209	1,537	1,842	216
Seeds of oilseed rape with erucic acid < 2%, also chipped	197,577	233,868	218,564	48,863
Seeds of oilseed rape with erucic acid =>2%, also chipped	11,053	15,953	20,443	2,065
In total	209,839	251,358	240,849	51,144

We also requested information about the Austrian Federal States ("Bundesländer") to which the imported OSR seeds were transported, about oil mills, the purchasers and processers and - as already discussed before - about registered imported OSR varieties from abroad. However, Statistik Austria informed us that because of data privacy reasons these data were not available for the present project. To get information, we identified contact data of Austrian oil mills with Herold, the classified directory ("Branchenbuch") and WKO (https://www.wko.at/). For first information we visited their homepages in the internet. Several identified oil mills were then asked for detailed information such as if they import OSR for processing from abroad (not shown because of privacy reasons, but provided to the Federal Ministry of Health as supplementary information). The authorised staff of the oil mills also did not make information available about single OSR varieties they purchase from abroad.

All contacted mills agreed on the fact that the purchased seeds in all cases are usually delivered in bulk mixtures without separate assignment to defined OSR varieties. For example, the manager of the oil mill Raab in Upper Austria told us that they receive organic OSR seeds from Romania as a bulk mixture only complying with the quality standards (organic production, absence of GM material, oil content, content of erucic acid and glycosinolates) and again without assignment to certain varieties. The oil mills provided different more or less detailed data of amounts of imported OSR. Beyond that, also authorised managers for transportation of oilseeds from the OEBB (e.g. switchyard Kledering) as well as from loading ports along the River Danube (e.g. Krems, Enns) confirmed that the common form of trading and handling of purchased OSR are bulk mixtures not being assigned to certain varieties but to quality standards. Train loads are also just marked as “oilseeds” (personal communication: R. Steiner, OEBB).

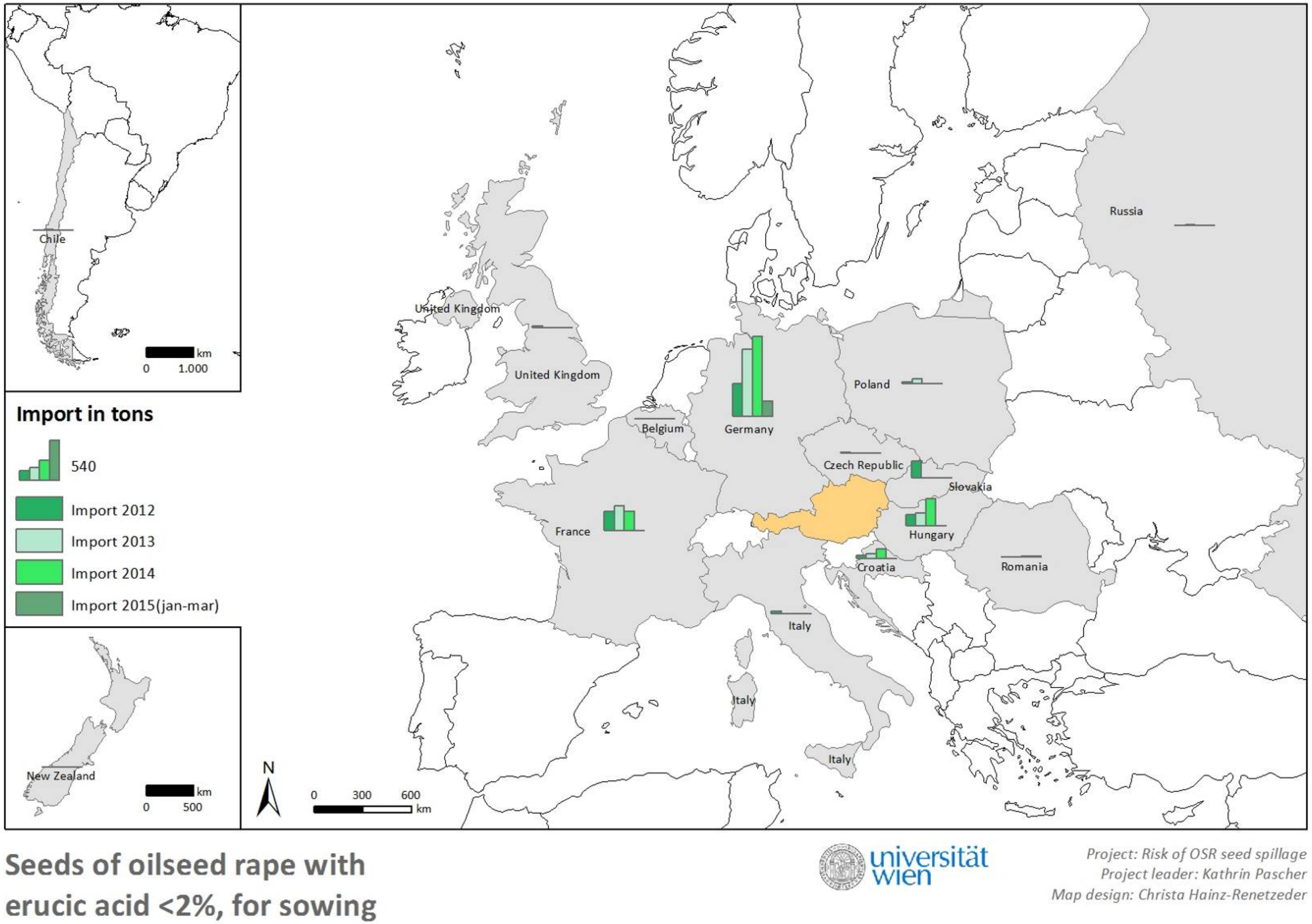


Figure 6: Countries exporting OSR seeds for seeding to Austria in the years 2012 until 2015. Source: Statistik Austria.

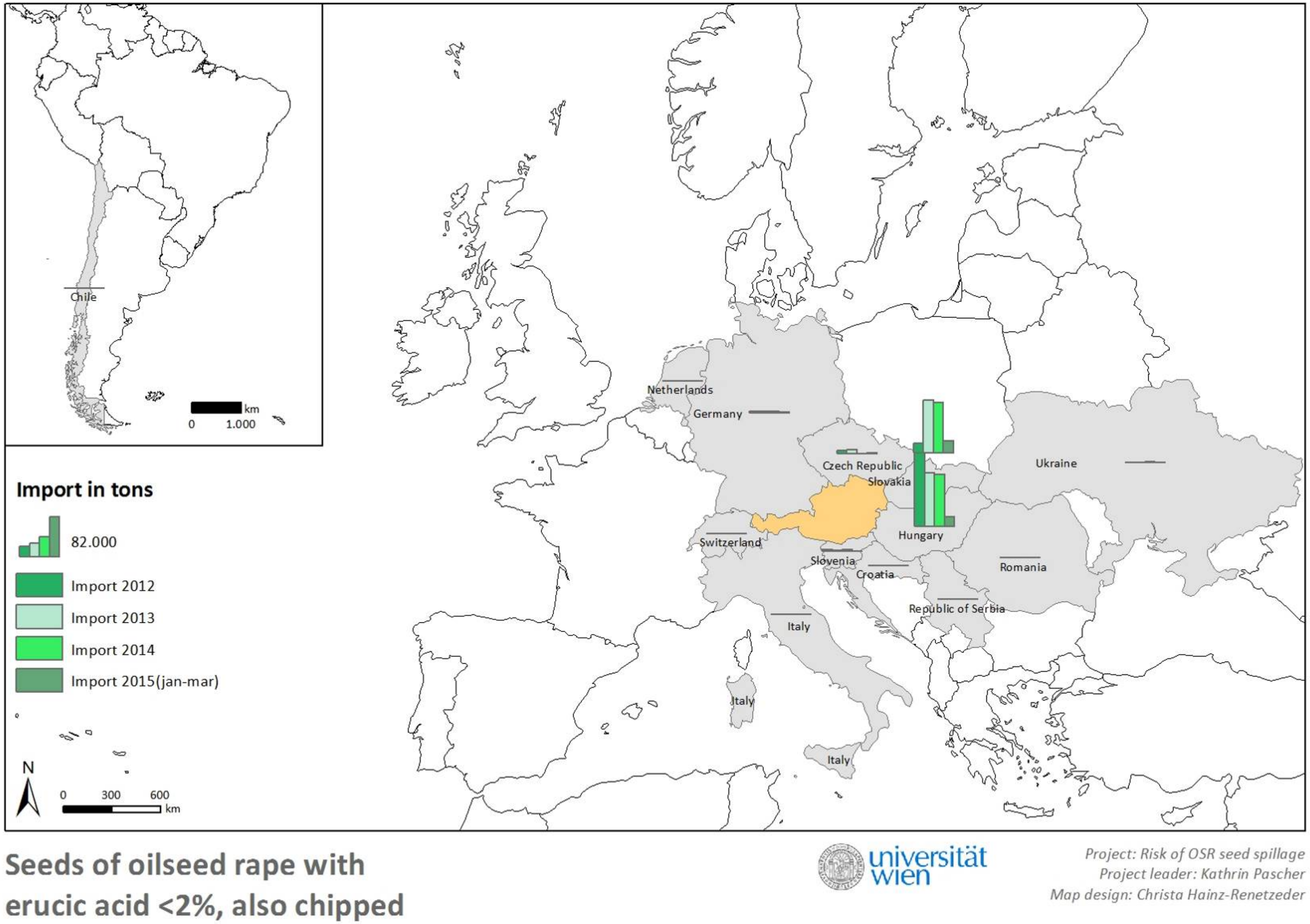


Figure 7: Countries exporting OSR seeds with low erucic acid content to Austria in the years 2012 until 2015. Source: Statistik Austria.

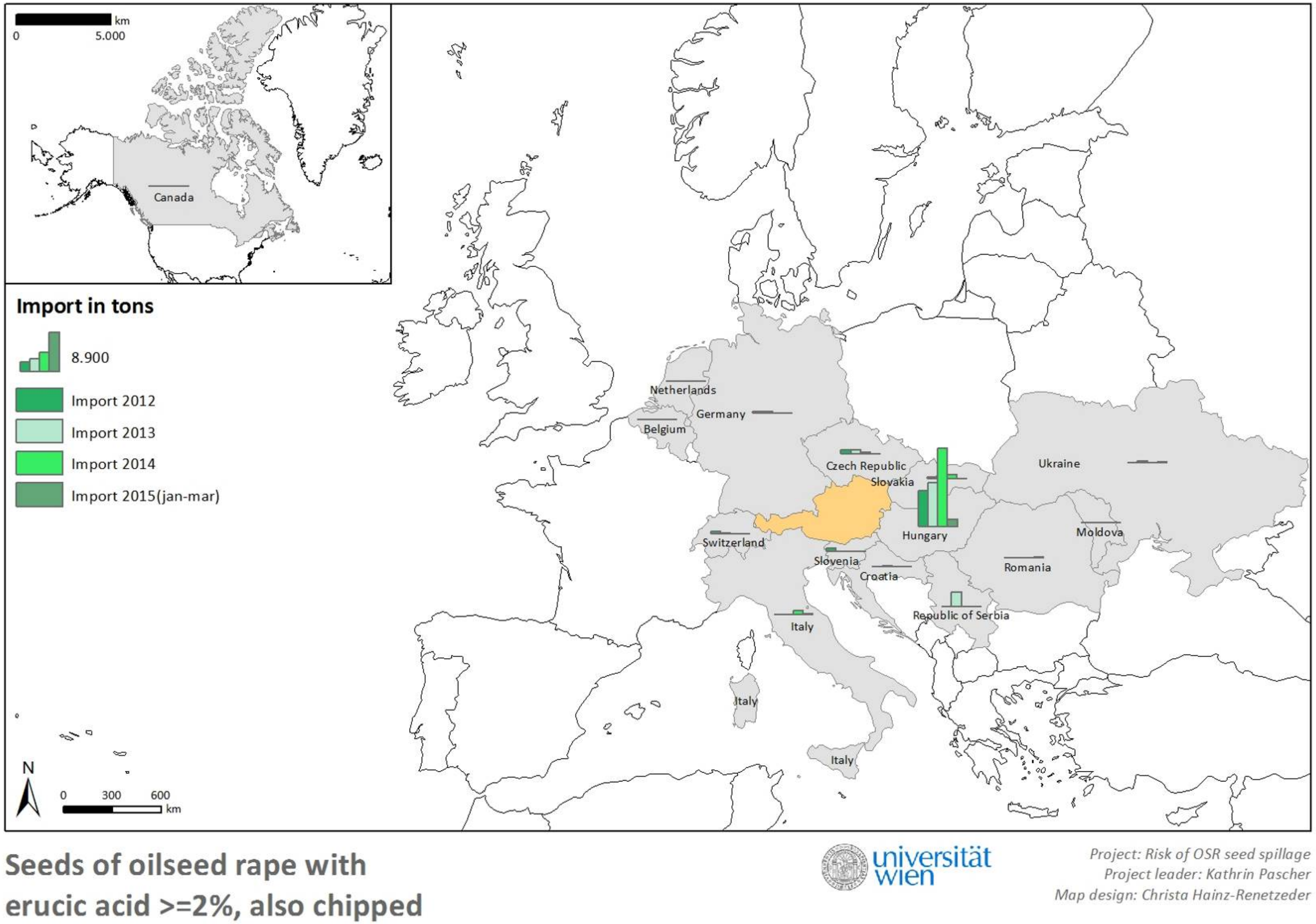


Figure 8: Countries exporting OSR seeds with high erucic acid content to Austria in the years 2012 until 2015. Source: Statistik Austria.

6.1.2. Transportation of imported OSR to and within Austria

Data concerning main transportation routes on which OSR is imported are valid and non-transparent. In principle, three possible ways for transportation can be identified:

- ship
- railway
- road.

According to the data of Statistik Austria, all three OSR transportation alternatives are in use, a fact which was also confirmed during fieldwork in Austria by the staff of relevant institutions (e.g. OEBB, oil mills - e.g. Bunge, ports - e.g. Albern, Krems, Enns). However, detailed data concerning mode of OSR transportation are not recorded. For instance, driving routes for transportation which truck drivers use are neither predefined nor registered by the oil mills or ports and therefore are not retraceable. They may be individually chosen by the drivers. OSR is included in the wider category of oilseed feedstock without specific proportions of OSR identified in the dataset.

Statistics on transport do not indicate whether the good stays within Austria or is going to be transported abroad. Statisticians speak of "receiving of goods". These two mentioned major drawbacks have to be kept in mind, when looking at transportation statistics.

6.1.2.1. Shipping

Up to 2011, the category *"oilseed, oleiferious/oleaginous fruits and fats" ("Ölsaaten, Ölfrüchte und Fette")* included OSR seeds. Since 2012, amendments based on EU-guidelines lead to new classifications of goods. At this time, OSR was incorporated into the category of goods *"other products of vegetable origin" ("Andere Erzeugnisse pflanzlichen Ursprungs"*) together with crop straw and husk, soy beans, peanuts, cotton, oilseed, tobacco, fiber plants, forage plants, plants for production of beverages, seeds of beat and forage crops, raw herbal agents, spice plants, plants for aromatic, narcotic and pharmaceutical purposes as wells as other perennial plants.

Survey of inland waterway transportation on the Danube is conducted as complete inventory count and includes all ships - inland and foreign - which transport goods on the Austrian section of the River Danube. Discharge weights are given separately only for the large ports along the River Danube. Small ports are grouped into one category because of reasons of confidentiality.

In 2011, 19,865 tons of *"oilseed, oleiferious fruits and fats"* were transported on the River Danube and unloaded at the port of Enns, which is 0.36% of total discharge in Austria of this category of goods. The main countries from which imports to Austria were received, were Bulgaria with 72% of total discharge (14,397 tons) followed by Hungary with 3,267 tons (16%) and Switzerland with 1,194 tons (6%, Table 4).

In the years 2012 to 2014, the main country which exported OSR to Austria was Hungary with 67% (2012), 36% (2013), and 59% (2014) of *"other products of vegetable origin"*, followed by Germany and the Netherlands (Figure 9; Table 5). Similar to all imported shipping cargo, this category of goods amounted to 3.2% (2012), 3.7% (2013), and 3.8% (2014). The largest ports in Austria received nearly no discharge of this kind of goods. Only the smaller ports were the main reloading points. Because of changing the category assignment of goods from the year 2012 onwards, a direct comparison of discharge data over time is not possible. Nevertheless, a few facts can be noticed. It can be assumed that about 8-10% of the products in the new category might be oilseeds. In 2012, there was only small discharge in Enns from Bulgaria and none from Switzerland which were the main countries of OSR export to Austria in 2011 (Table 2).

Table 4: Discharge of products in Austrian ports along the River Danube listed by importing countries. In 2011, OSR was included into the category of goods of "oilseed, oleiferious fruits and fats". Amount is given in tons.

	2011
	Port of Enns
Germany	687
Belgium	-
Netherlands	-
Switzerland	1.194
Ukraine	-
Bulgaria	14.397
Hungary	3.267
Serbia	330
Total	19.875

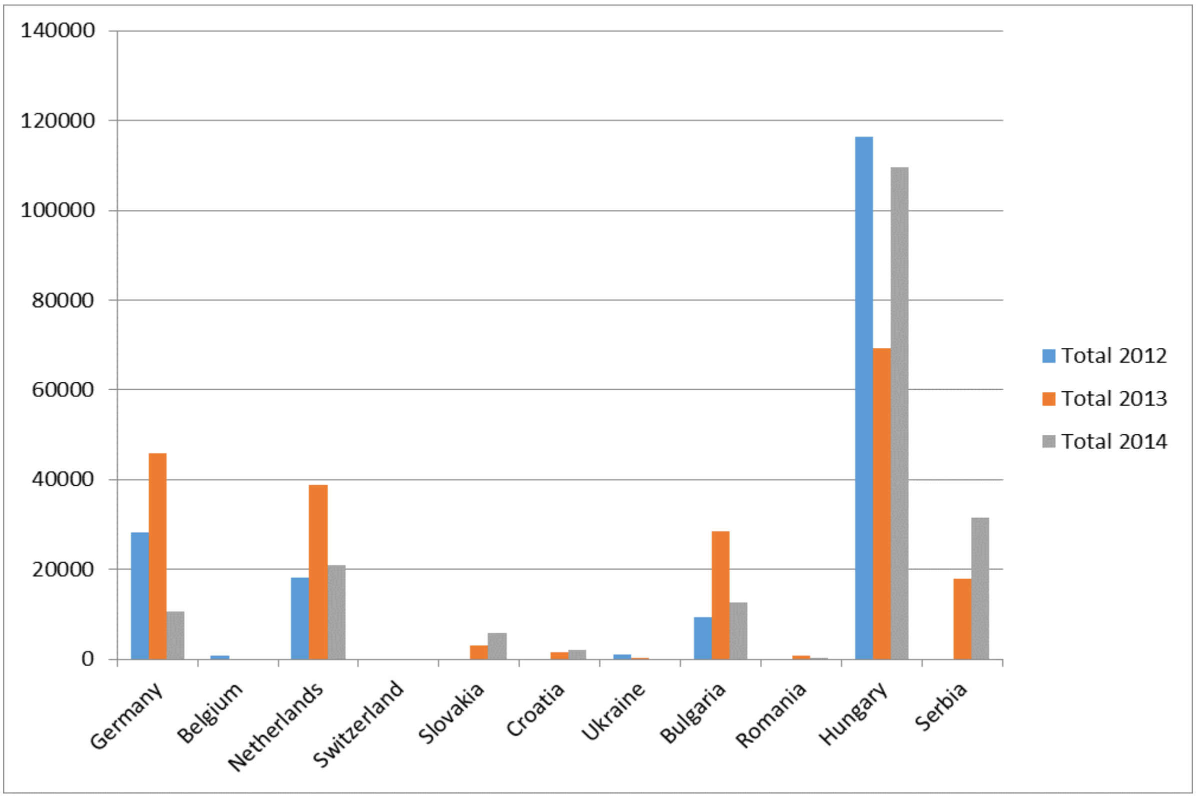


Figure 9: Amounts of OSR seeds in tons exported to Austria from the listed European countries and the Ukraine in 2012, 2013 and 2014.

Table 5: Discharge of products in Austrian ports along the River Danube listed by importing countries. From 2012 onwards, OSR was assigned to the category of goods *"Other products of vegetable origin"*. Amount is given in tons.

2012	Port of Krems	Port of Enns	other ports	
Germany	-	-	28,332	
Belgium	-	-	858	
Netherlands	448	-	17,639	
Switzerland	-	-	-	
Ukraine	1,029	-	-	
Bulgaria	-	3,268	6,081	
Hungary	4,298	323	111,739	
Serbia	-	-	-	
Total				174,015

2013	Port of Krems	Port of Enns	other ports	
Germany	7,626	-	38,278	
Netherlands	-	-	38,743	
Slovakia	-	-	3,068	
Croatia	-	-	1,540	
Ukraine	349	-	-	
Bulgaria	-	15,508	12,933	
Romania	-	-	766	
Hungary	2,187	4,191	63,012	
Serbia	-	-	17,827	
Total				206,028

2014	Port of Krems	Port of Enns	other ports	
Germany	-	625	9,889	
Netherlands	-	-	20,886	
Slovakia	-	-	5,937	
Croatia	2,004	-	-	
Bulgaria	-	9,263	3,452	
Romania	290	-	-	
Hungary	1,179	6,363	102,003	
Serbia	187	-	31,272	
Total				193,350

6.1.2.2. OSR transport *via* railway

Statistics Austria is generating a complete inventory count of railway cargo as transported by the Austrian Federal Railway Company OEBB. Available data are grouped according to countries of import, custom offices and unloading provinces. Again, OSR is not listed as an own category but assigned to the category of goods “oilseed, oleiferious fruits and fats”. In total, nearly 30 million tons of cargo were transported *via* railway to Austria in the year 2012, whereas the amount of oilseed added up to 162,421 tons which is 0.541% of railway transport. In comparison, even less amounts were transported in the years 2013 and 2014 (87,911 tons = 0.33 and 53,683 tons = 0.20% of railway transport respectively).

Most of the category “oilseed, oleiferious fruits and fats” - which as mentioned before also included OSR - was imported from Germany, followed by the Netherlands and the Czech Republic (Table 6) in the year 2012. In 2013, Germany was followed by Hungary and the Netherlands (Table 7) and in 2014, most of the imported OSR originated from Czech Republic followed by Germany and Romania (Table 8). As Figure 10 clearly shows, the overall amount of imported OSR decreased enormously over the years.

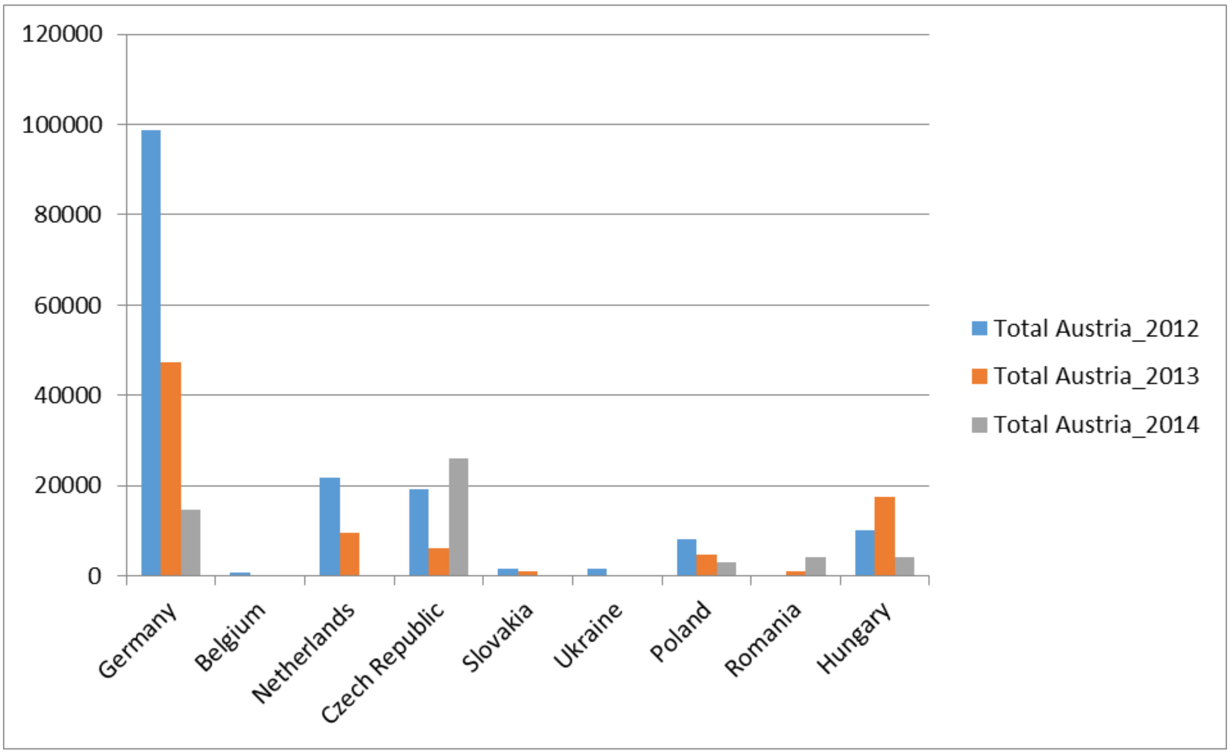


Figure 10: Import of the category of goods “oilseed, oleiferious fruits and fats” to Austria by importing countries. Amount is indicated in tons.

In 2012, Eastern countries and Germany transported oilseeds mainly to Vienna, whereas Belgium and the Netherlands delivered mainly to Lower Austria and other Federal States (Figure 11). Romania was the main country to export OSR to Upper-Austria in 2013 and 2014. Mainly Germany transported goods to other Federal States than Vienna (Figure 12, Figure 13).

Table 6: Import of the category of goods *"oilseed, oleiferious fruits and fats"* to Austrian Federal States *via* railway in 2012. Amounts are indicated in tons.

	Carinthia	Upper Austria	Lower Austria	Styria	Vorarlberg	Vienna	Total Austria
Germany	3,111	1,919	926	1,555	57	91,235	98,803
Belgium	-	-	701	-	-	-	701
Netherlands	-	-	10,917	4,615	-	6,313	21,845
Czech Republic	-	1,997	-	-	-	17,307	19,304
Slovakia	-	-	-	-	-	1,585	1,585
Ukraine	-	-	-	-	-	1,639	1,639
Poland	-	-	-	-	-	8,285	8,285
Hungary	-	1,394	409	-	-	8,456	10,259
Total import	3,111	5,310	12,953	6,170	57	134,820	162,421

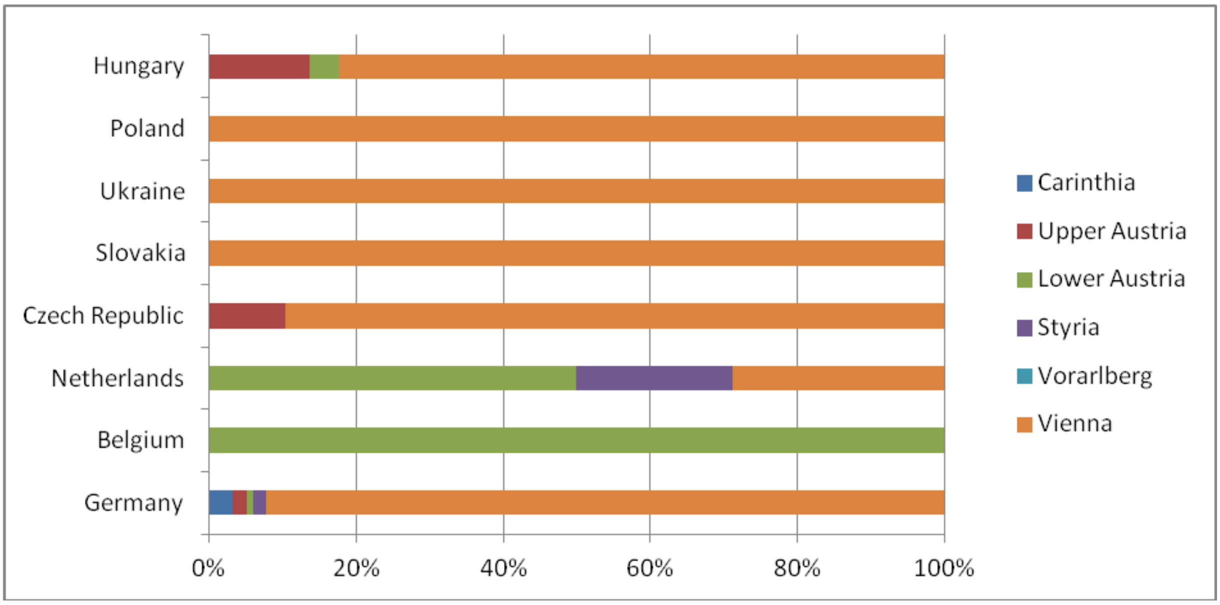


Figure 11: Import of the category of goods "oilseed, oleiferious fruits and fats" to Austrian Federal States in the year 2012 *via* railway given in proportions listed by countries of origin.

Table 7: Import of the category of goods *"oilseed, oleiferious fruits and fats"* to Austrian Federal States *via* railway in 2013. Amounts are given in tons.

	Carinthia	Upper Austria	Lower Austria	Styria	Vienna	Total Austria
Germany	1,342	0	9,470	2,442	34,093	47,347
Netherlands	0	0	3,594	2,184	3,932	9,710
Czech Republic	0	0	1,247	0	5,058	6,305
Slovakia	0	0	0	0	1,005	1,005
Poland	0	0	0	0	4,883	4,883
Romania	0	1,023	0	0	0	1,023
Hungary	0	0	0	0	17,638	17,638
Total import	1,342	1,023	14,311	4,626	66,609	87,911

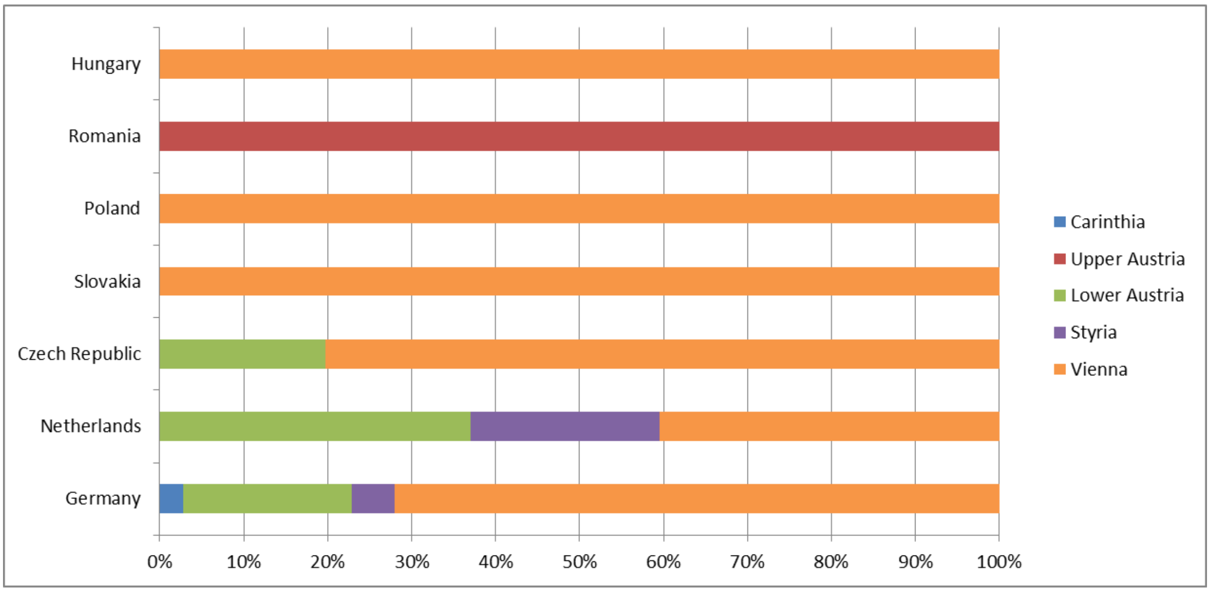


Figure 12: Import of the category of goods “oilseed, oleiferious fruits and fats” to Austrian Federal States in the year 2013 *via* railway indicated in proportions listed by countries of origin.

Table 8: Import of the category of goods *“oilseed, oleiferious fruits and fats”* to Austrian Federal States *via* railway in 2014. Amounts are given in tons.

	Carinthia	Upper Austria	Styria	Vienna	Total Austria
Germany	609	0	3,530	10,426	14,565
Czech Republic	0	0	0	26,088	26,088
Poland	0	0	0	2,997	2,997
Italy	0	0	0	1669	1,669
Romania	0	4,270	0	0	4,270
Hungary	0	0	0	4,094	4,094
Total Import	609	4,270	3,530	43,605	53,683

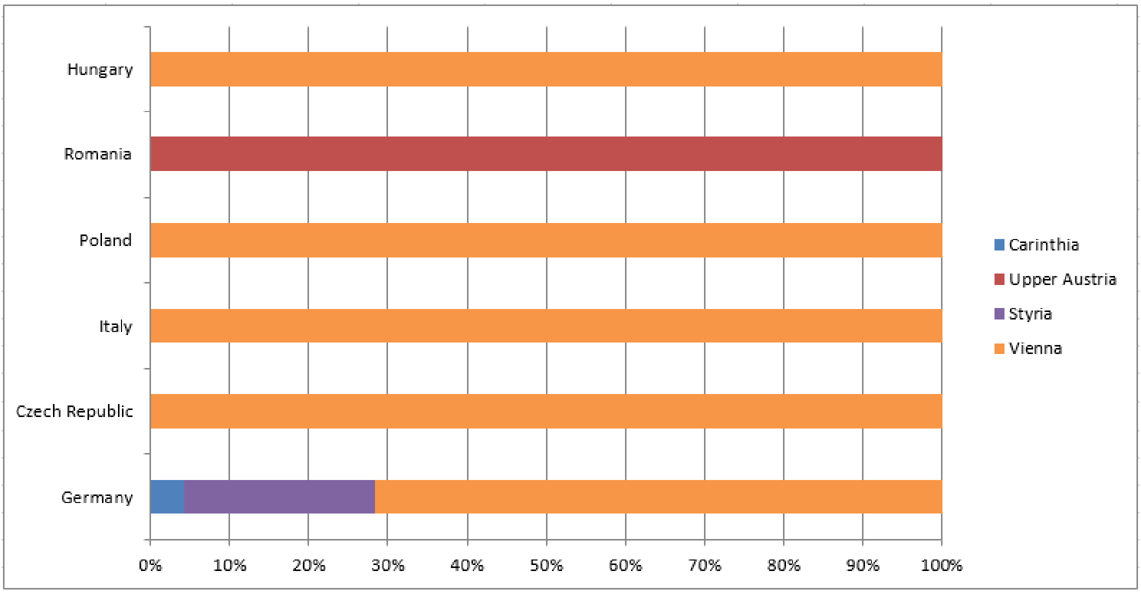


Figure 13: Import of the category of goods “oilseed, oleiferious fruits and fats” to Austrian Federal States in the year 2014 *via* railway in proportions listed by countries of origin.

Every carriage *via* railway coming from bordering countries must define and declare its frontier crossing point (“Schienengrenzübergang”) which will be used for transportation to Austria. Since 1995, no checks at custom offices are conducted anymore because of the accession of Austria to the European Union (Table 9). These crossing points do not necessarily correspond to train stations on the Austrian side of the border. Their locations can be found on the official infrastructure map of the Austrian Federal Railways (OEBB; http://www.oebb.at/infrastruktur/de/_p_3_0_fuer_Kunden_Partner/3_2_Schienennutzung/3_3_Schieneninfrastruktur/3_3_6_Karten/02_DMS_Dateien/Infrastrukturnetzuebersichtskarte.jsp).

Table 9: List of Austrian frontier crossing points (Schienengrenzübergang) as defined by Statistik Austria.

Nr.	Name of station
495	Arnoldstein-Bahnhof
485	Bleiburg
995	Bregenz-Lindau-Reutin
996	Bregenz-Lindau-Stadt
896	Brenner-Bahnhof
886	Ehrwald-Bahnhof
988	Feldkirch-Buchs
285	Gmünd-Bahnhof
990	Höchst-St. Margarethen
385	Hegyeshalom / Nickelsdorf
295	Hohenau
394	Jennersdorf
321	Kittsee
389	Sopron-Loipersbach, Deutschkreutz
390	Sopron-Raaberbahnhof / Baumgarten (Raaberbahn; does not belong to OEBB)
890	Kufstein
289	Marchegg
386	Pamhagen
590	Passau
290	Retz
490	Rosenbach
685	Salzburg-Bahnhof
887	Scharnitz-Bahnhof
888	Scharnitz-Mittenwald
895	Sillian-Bahnhof
585	Simbach-Braunau-Bahnhof
790	Spielfeld-Bahnhof
595	Summerau
402	Villach
885	Vils-Bahnhof

Passau in Germany was the frontier crossing point where most of the cargo was declared, followed by Hohenau in Lower Austria (border to Czech Republic) and Hegyeshalom in Hungary / Nickelsdorf in Austria (Table 9; Figure 14). Only small amounts of cargo were declared at the remaining frontier-crossing points.

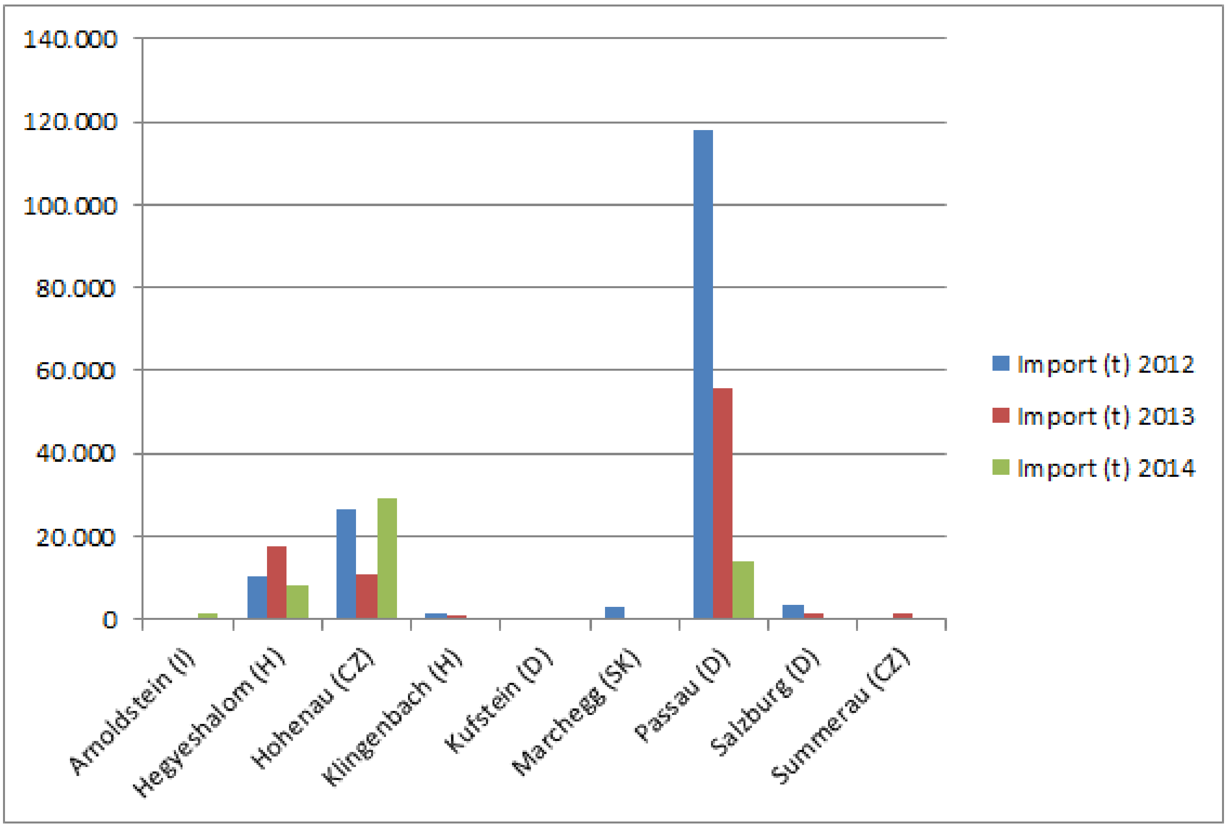


Figure 14: Imported OSR declared at the listed frontier crossing points in the years 2012, 2013 and 214.

6.1.2.3. Road carriage

In contrast to information on shipping and railway cargo, the survey on road carriage is based on random sampling of Austrian transport companies only. Foreign freight carriers are not sampled in any way by Statistik Austria and therefore, no import activity of OSR by foreign companies is registered. The amount of OSR seed which is imported to Austria *via* roads is inestimable. Statistics at this level of detail bear a too large uncertainty that any reliable conclusion could be drawn.

7. Materials & Methods

7.1. Basic data for the selection of sample sites

As far as possible, usable layers for main roads, railway network (Figure 15) and river courses (Figure 16) of Austria were organised as a basis for our sampling procedure. An OSR cultivation map based on IACS cultivation data of 2012 for the Austrian municipalities was created (see Figure 1). Climatic data were not used for the stratification procedure.

Oil mills in Austria were identified using information of the study of Reiner (2006) and actualized. Afterwards, the managers of these processing facilities were contacted. Because of confidentiality concern detailed information is provided as supplementary material for the Austrian Ministry of Health only.

Interviews and additionally E-mail enquiry were conducted addressing the following issues:

- Does the contacted oil mill actually process OSR seeds?
- Does the oil mill use imported OSR for processing?
- If yes, which are the importing countries?
- How large are the imported amounts in tons per year?
- Which transportation routes are usually used especially from the truck-drivers from abroad to deliver the oil mill.

In accordance with Statistik Austria also the oil mills gave the information that they do not register specific OSR varieties which are imported to their mills for processing. The seeds are delivered in bulk mixtures of several OSR varieties. Again, the quality standards are also defined complying with oil content, content of erucic acid and glycosinolates as well as absence of GM material. A table (not shown in the report, but part of the supplementary) of 24 oil mills was created including contact address and all the inquired information, 18 of the OSR processing facilities actually process OSR, four of these import OSR from abroad.

Based on the information from Statistik Austria from which country to which Austrian Federal State OSR was imported, the main transportation routes were identified using route planner (Google Maps, default parameters), inserted into GIS and marked in a map (Figure 17). Furthermore, the railway net for transportation throughout Austria was identified based again on information of the Statistik Austria including the locations of railway stations.

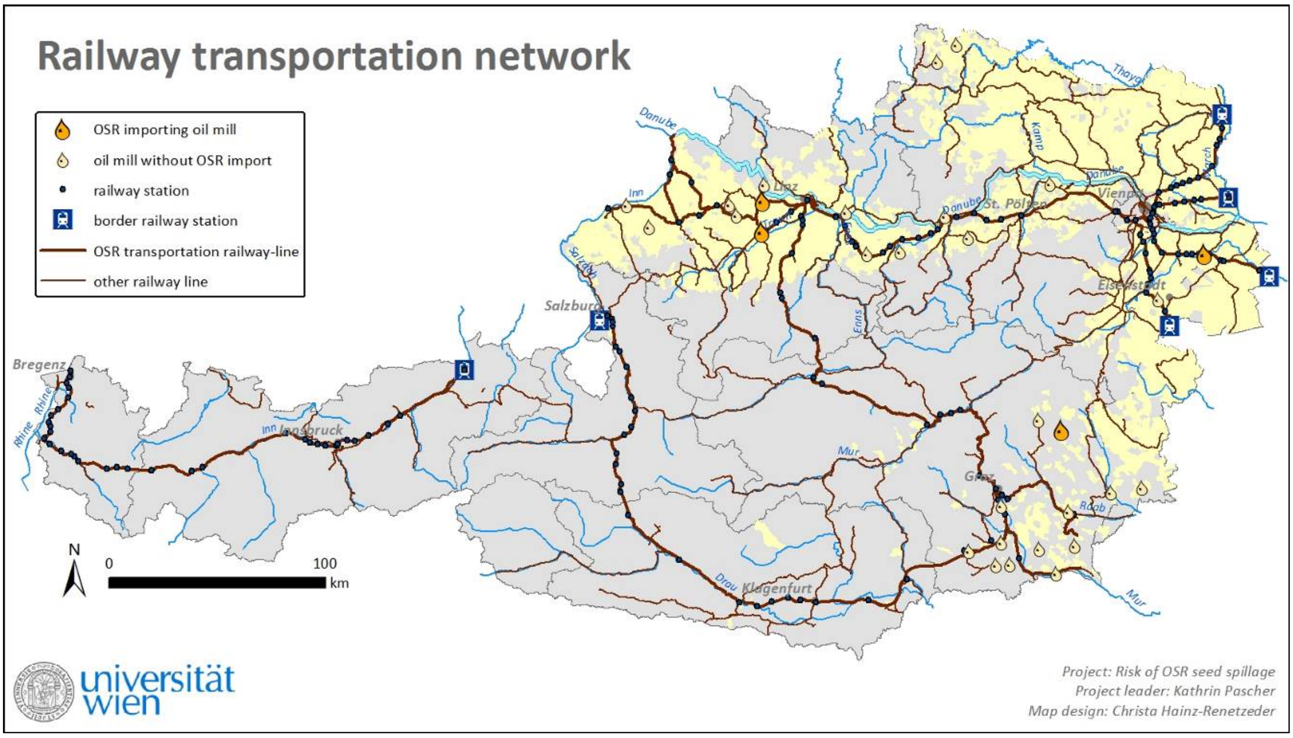


Figure 15: Main railway transportation network through Austria. Train stations along identified OSR transportation routes are marked with blue spots. Small railroads were not included. Light yellow: OSR cultivation areas.

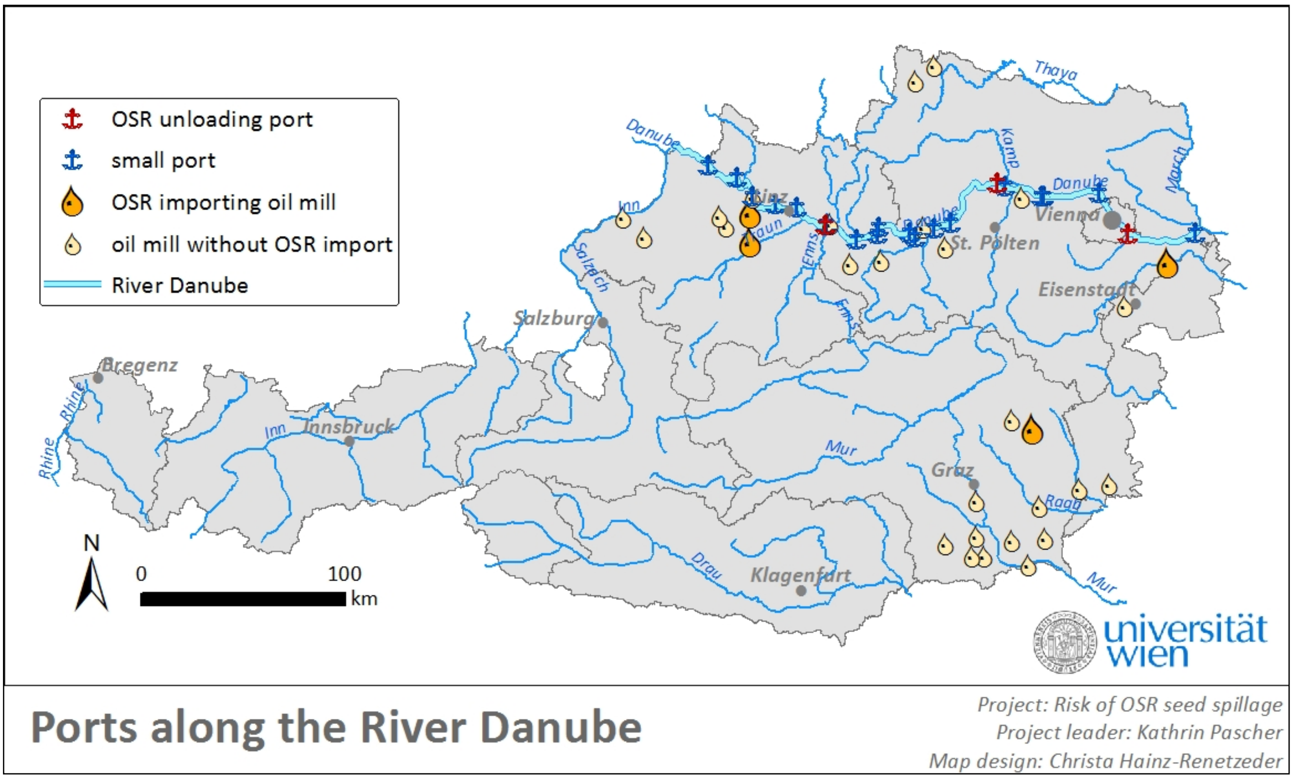


Figure 16: Main river courses including ports and location of oil mills in Austria which were contacted for the present study.

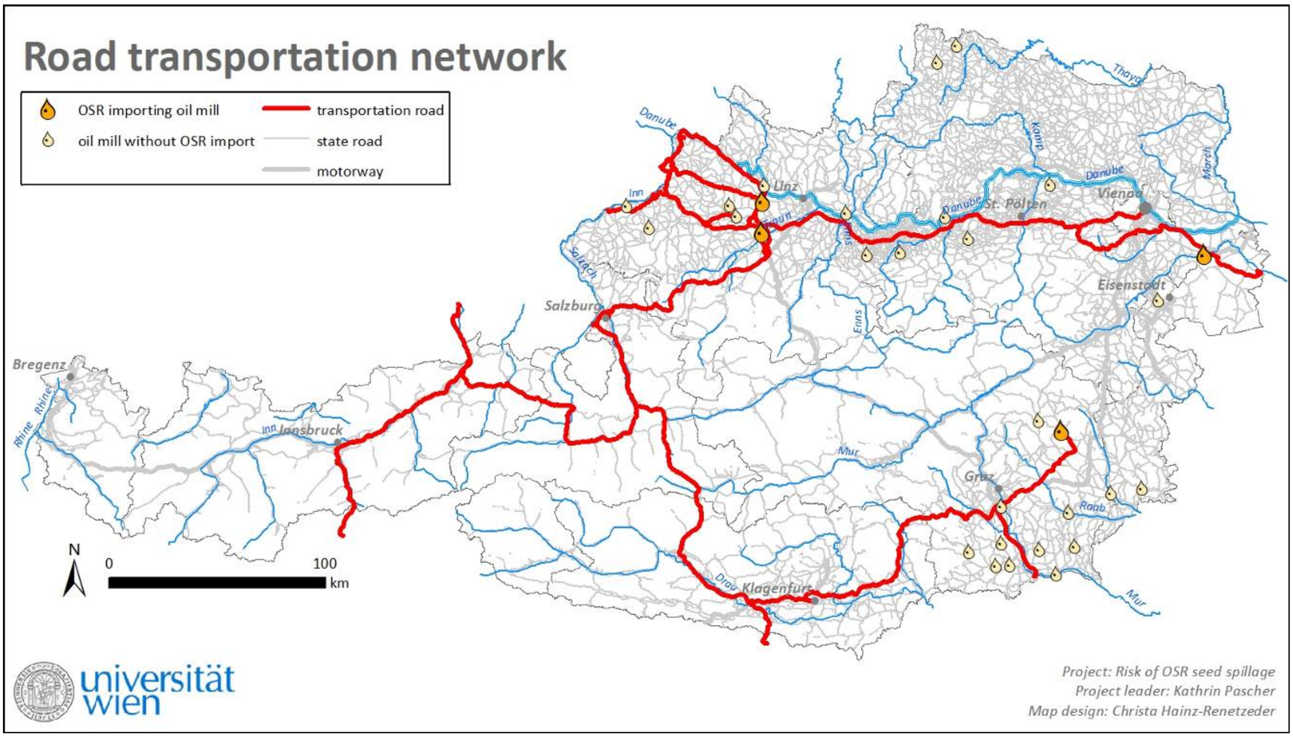


Figure 17: Main transportation roads of OSR and other goods in Austria and location of oil mills which were contacted for the present study.

7.2. Sampling procedure

Similar to the study of HECHT et al. (2013) sampling focused on two main approaches – predefined hotspots as well as randomly chosen sites:

- **Predefined hotspots:** sample sites with a high expectation for seed spillage such as switchyards (freight railway stations), border railway stations, main ports, and OSR importing oil mills in Austria such as the oil mill Bunge.
- **Random sampling of hotspots:** railway stations, small ports, small oil mills, and oilseed processing facilities.
- **Random sampling of transport road sections:** Transportation roads (state roads; in German language "Schnellstraßen") also directly starting from the exits of motorways and smaller roads which act as distribution roads to oil mills were partitioned into 2,000 m sections. From all selected sections (22) a random sample (30-50) or even all feral plant individuals were collected depending on the respective present plant numbers.

Information, for instance of the Statistik Austria, Austrian seed breeders, and OSR processing facilities such as oil mills was used to identify hotspots of seed spillage. For this analysis the focus was laid upon the largest OSR processing facilities in Austria such as the oil mill Bunge in Bruck an der Leitha and their main railway supply lines and roads from the Austrian border. The western and southern parts of Austria, where no OSR cultivation is recently performed, were also sampled for reference reasons such as the Inn Valley or several road sections in Salzburg. Figure 16 shows the location of oil mills in Austria, distinguished between importing and not importing mills based on the received information. Unloading ports along the River Danube are marked in red, smaller ports in blue. For the sampling procedure three types of sites were not considered in the sampling procedure: sampling in motorway section as well as railway line sections would have caused an enormous effort in obtaining permits for this work. Moreover, sampling along these sites would have been very dangerous. The sampling experience in the Swiss study of HECHT et al. (2013) showed that it was often necessary to change the randomly selected sections along railways because of inadequate accessibility and safety reasons (e.g. railway tubes, bridges, railway curves). But some of the selected 2,000 m road sections included exits of motorways. Furthermore, we sampled ports along the River Danube as hotspots of seed spillage because of loading activities, but no river course sections.

The sampling procedure focussed on the selection of the following sites (see Figure 17, 18 and Table 14):

- Relevant railway stations on the border of EU countries which import OSR to Austria (GBH): predefined selection: 7
- Switchyards (FB): predefined selection: 2
- Railway stations within OSR cultivation areas (BHR): random sampling: 10
- Railway stations outside OSR cultivation areas (BHK): random sampling: 10
- Ports along the River Danube (DH): 6
  - Main OSR loading ports (DH): predefined selection: 3 (Albern, Enns, Krems)
  - Small ports (DH): random sampling: 3
- Main transportation roads within OSR cultivation areas (SR): random sampling: 11
- Main transportation roads outside OSR cultivation areas (SK): random sampling: 11
- OSR importing oil mills (OEM): predefined selection: 3 (Bunge, Fandler, Raab)
- Processing company (OEM): predefined selection: 1 (VFI Wels)

In total: 61 sample sites

Because there is no corresponding railway station of Passau in Austria we visited the railway station in Passau in Germany. According to the data of the Statistik Austria which is the official information office, Passau was the main frontier crossing point where 73% (nearly 118,000 tons, see Figure 14) of OSR cargo were declared in 2012. After contacting the officer of the railway station in Passau we were told that there is no corresponding railway station in Austria. For this reason this sample site had to be dropped. Hence, the sampling plan includes altogether 60 sample sites which were sampled twice, spring and early summer 2014 and 2015 (Figure 18, details Upper Austria: Figure 19).

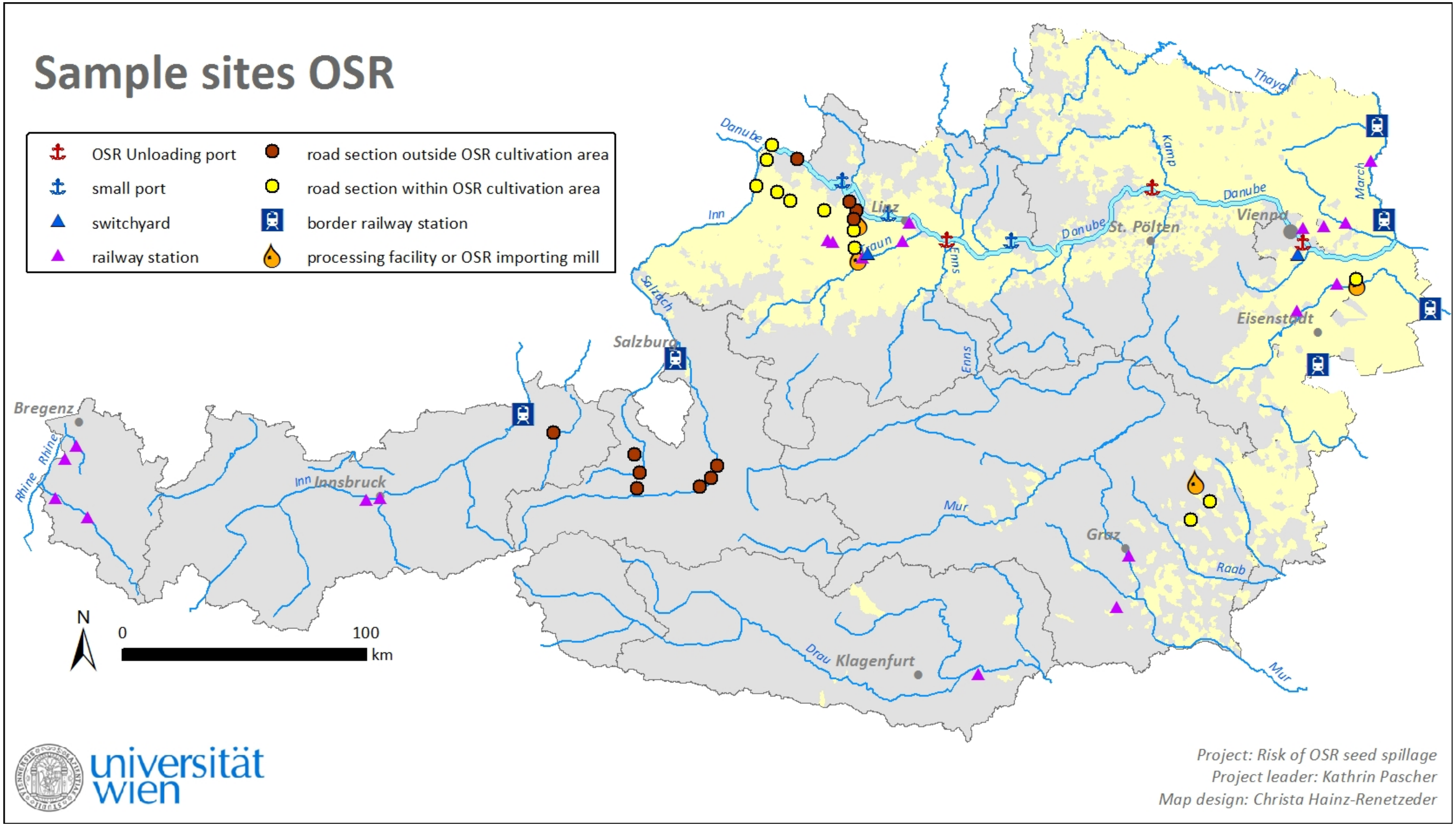


Figure 18: Location of the 60 sample sites. Border railway stations (GBH): 6, switchyards (FB): 2, railway station within OSR cultivation areas (BHR): 10, railway station outside OSR cultivation areas (BHK): 10, ports along the River Danube (DH): 6, main transportation roads within OSR cultivation areas (SR): 11, main transportation roads outside OSR cultivation areas (SK): 11, OSR importing oil mills (OEM): 3, processing facility (OEM): 1.

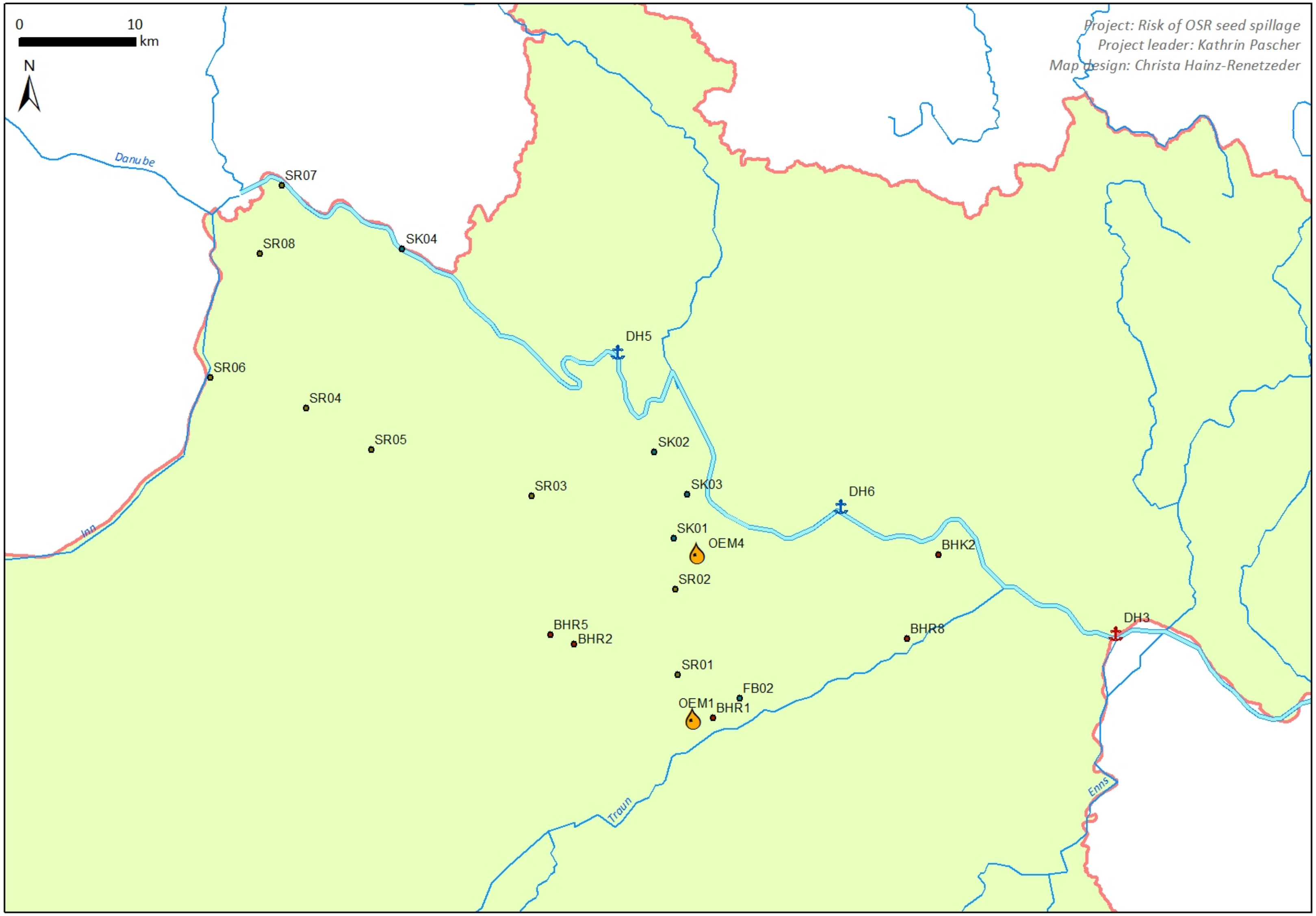


Figure 19: Detailed map completing Figure 18. For better overview the location of sample sites in Upper Austria.

7.3. Fieldwork – Sampling of feral oilseed rape plants

All 60 sample sites were visited using GPS and detailed OpenStreetMaps (OSM) which were adjusted for the present project (Figure 20). In compliance with OEBB (Österreichische Bundesbahn, Austrian Federal Railways) safety regulations, two training courses (SIG1 and SIG2) had to be attended in Leoben (Styria) and examinations had to be passed. This is the standard requirement to get permission from the OEBB to work in “non-public areas” of the railway which was necessary for sampling of feral OSR plants in the surroundings of railway stations.

Since, safety requirements are very strict, working permission for each single station had to be obtained from each railway station manager (“Stationsvorsteher”) for sampling at the railway stations and switchyards. Sampling of plants directly on the tracks had to be accompanied by a railway station manager on duty in any case. This unexpected procedure required complex planning and time effort, but was necessary to perform the sampling under absolute safe conditions.

Young leaves of the feral OSR individuals were collected. For each individual the leaves were stored in a tea bag. Five tea bags were put together in a small plastic bag und filled with silica-gel for drying, a ubiquitously used approach for field preservation of leaf samples for DNA studies (Chase & Hillis 1991). Hence, high temperature especially in the car during field work could not cause destruction of plant material. Careful labelling was performed on the tea bags as well as on the plastic bags including the sampling location, its synonym, the population ID and the individual number. Additional information was recorded on a survey sheet (see next chapter: implementation of a sampling database). GPS data in most cases even for individuals were taken, in other cases when the plants were situated directly side by side or arising in large amounts, the position of the first and the last plant of a population were recorded. These waypoints were marked in the used maps of the sample sites. For example, the port of Albern is shown in Figure 21.

2,113 feral OSR plants were sampled in accordance with the Swiss studies (Hecht et al. 2013). As far as possible depending on permission and accessibility, sites located in the same region were sampled continuously one after each other on long fieldwork days up to 15 hours for high efficiency in working and traveling time as well as traveling costs. Because of the delayed receipt of important data e.g. from the Statistik Austria, training courses obliged from the OEBB, permissions which we had to obtain in nearly every case and maintenance work of the oil mill Bunge, sampling had to be conducted over a longer time span as originally planned. Hence, fieldwork was prolonged and therefore performed from April, 22th until August, 19th in 2014. In 2015, fieldwork was done from April, 23th until June, 25th.

7.4. Implementation of a sampling database

An Access database was implemented conforming to the compiled survey sheet for field work. The following information was recorded during field work and filled into a survey sheet:

- Site number, location, date of sampling, photo numbers
- Characteristics of the population: population ID, size of population, number of individuals sampled, new or old establishment of the population, stage of maturity (blossoms, seeds, etc.), constitution and pests infestation
- Location of the population: GPS coordinates, GPS waypoints
- Hybridisation partners of OSR: occurrence and frequency in sample sites
- Additional comments and observations.

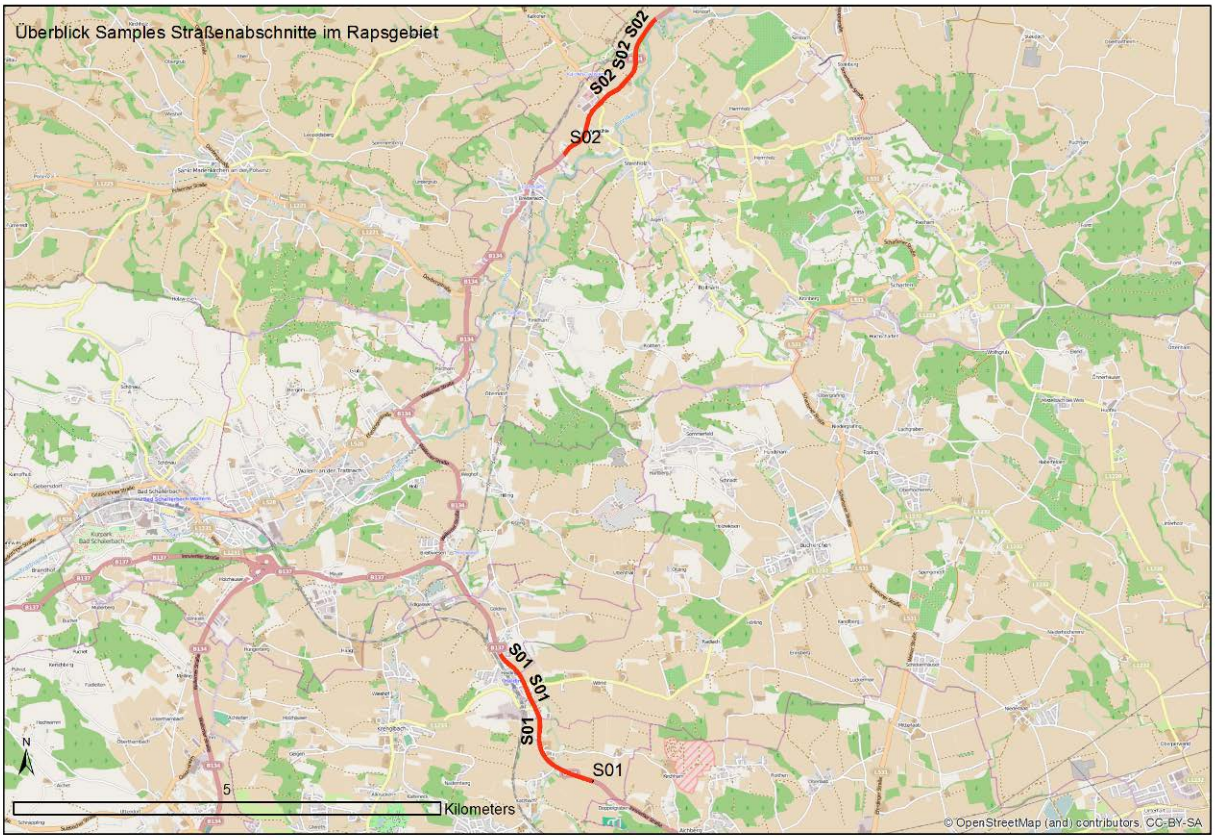


Figure 20: OSM maps and aerial photographs of two road sections within the Austrian OSR cultivation area SR01 und SR02 as examples for the sampling support. The two red marked sections of the roads indicate the sampling area of altogether 2 kilometres on each road side. Data source: www.basemap.at.

Figure 21: Waypoints indicate the positions of sampled feral OSR plant populations. Port of Albern on the River Danube; data source: www.basemap.at.

7.5. Provision of OSR variety seed samples (“Sortenmuster”)

In Austria, the Austrian Agency for Health and Food Safety (AGES – Agentur für Gesundheit und Ernährungssicherheit) is the official authority for the admission of OSR varieties. After contacting, we received links to relevant websites:

- Feldanerkennungsflächen: http://www.baes.gv.at/saat-pflanzgut/statistiken/
- Hauptsorten und Vermehrungsflächen: http://www.baes.gv.at/pflanzensorten/oesterreichische-beschreibende-sortenliste/
- Österreichische Sortenliste (Austrian lists of OSR varieties): http://www.baes.gv.at/pflanzensorten/oesterreichische-sortenliste/.

Seed samples (so called “Sortenmuster”) of cultivated OSR varieties had to be requested from each of the Austrian seed breeders separately.

DI Helmut Reiner who was co-worker of the preceding OSR projects in Austria (Pascher et al. 2000, 2010) was contacted twice for his experience concerning information and procurement of OSR varieties grown in Austria but also from other to Austria importing European countries. H. Reiner also provided two OSR varieties (Honk and Mohican) which could not be received from the Saatzucht Donau because no variety seed samples were available any more. The two varieties had already been analysed in the project of Pascher et al. (2000) and used to be grown in Austria several years ago. After storing the seeds for a number of years, the germination capacity of these two varieties had already become low or even null. Unfortunately, germination attempts were repeated without success.

Four seed breeders in Austria were identified as the relevant breeders for OSR in Austria (personal communication from Johann Birschitzky, Saatzucht Donau):
The two main Austrian seed breeders are:

- Saatzucht Donau GesmbH & CoKG (Z, 124)
  The Saatzucht Donau GesmbH has OSR varieties on the Austrian market from own breeding (Z) as well as varieties from foreign partners.
  Raiffeisenware Austria (RWA AG, 89)
  RWA just works with foreign OSR varieties.

- Two smaller seed breeders active in Austria are:
  Pioneer Hi Bred Services GmbH (Z, 77)
  KWS Austria Saat GmbH (Z, 79).

Pioneer and KWS have breeding programs but not in Austria which means that the varieties are bred e.g. in Germany or France and are then registered and placed on the market in Austria.

7.6. Genetic analyses

Seeds of the following 49 listed main commercial OSR varieties were germinated in the Botanical Garden of the University of Vienna (see Table 10 and Table 12): Adriana, Alabaster, Albatros, Amethyst, Artago, Californium, Caracas, Carouser, Casoar, Castille, Columbus, Contact, Digger, DK Excellium, DK Exfield, DK Expertise, DK Expower, DK Exstorm, DK Sedona, DK Sequoia, Freddy, Gloria, Graf, Harry, Henry, Honk, Jimmy, Janus, Jolly, Kutiba, Ladoga, Lenny, Mickey, Mohican, NK Petrol, Orlando, Pedro, Peter29, Remy, Ricky, Sammy, Sherlock, Sherpa, Sidney, Solist, Tenno, Tommy, Top, and Vicking. Of those Columbus, Contact, Honk, Mohican, and Vicking did not germinate at all. As mentioned above, the varieties Honk and Mohican could not be provided from the Saatzucht Donau GesmbH & CoKG because these two varieties were used for growing in Austria several years ago, but are not in use anymore. Seed samples were organised from H. Reiner who still stored them from the preceding project Pascher et al. (2000). The variety samples are more than 15 years old and therefore, were not germinable anymore. For each of the remaining varieties, five individuals were harvested and dried in silica-gel except for the varieties Caracas, Henry, Tenno and Jolly, where due to poor germination only three individuals each could be harvested and dried in silica-gel. In addition to the samples from provided official commercial OSR varieties, seed samples from the warehouse Marchegg, the Oil Mill of Fandler and from the Oil Mill of Raab were germinated. With these seed samples we wanted to test an additional source of OSR varieties also for homogeneity of the purchased seed material. As mentioned before, in general the seeds are transported and used as bulk mixtures of several OSR varieties complying with quality standards for processing.

DNA of all 2,113 feral OSR plants sampled in 2014 and 2015 as well as in total 217 individuals of 45 out of 48 OSR varieties, totalling in 2,330 individuals (excluding replicate extractions) was extracted from 10 mg dried plant material - where possible - using the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. For each extraction plate, comprising 96 samples, on average three randomly chosen extraction replicates were included. Several of the feral plants dropped out during analysis because they could not be extracted due to too low amounts of leaf-material or to insufficient quality. The overall-number of samples with which amplification was tried, amounted to 2,116 individuals.

Seven SSR primers (Na12-A08, Na12-C06, Na-C08, Na12-C12, Na12-D11, Na12-E01 [amplifying two loci termed Na12-E01a and Na12-E01b], Na12-E06A) that amplify eight microsatellite loci were chosen, as they had been successfully used in previous studies (Pascher et al. 2000, 2006, 2010). To make full use of the used dyes (see below), an eighth primer pair was added. Based on Lowe et al. (2004) six primer pairs (Na10-B04, Na10-C01, Na10-C06, Na10-G06, Na12-H09, Na14-C12) were chosen out of 31 reported to be polymorphic in *Brassica napus*. Of those, Na10-C01 amplified all samples successfully and was therefore included in the analysis. PCR amplifications were done in two fourfold multiplexed reactions (primer labelling given in parentheses): the first reaction included Na12-A08 (6-FAM), Na12-C08 (VIC), Na12-C12 (NED), Na12-D11 (PET); the second reaction included Na12-C06 (6-FAM), Na12-E01 (VIC), Na12-E06A (NED) and Na10-C01 (PET). All labelled primers were obtained from Thermo Fisher Scientific (Waltham, USA). PCR amplifications were performed on a GeneAmp PCR System 9700 Thermocycler (Applied Biosystems, Foster City, USA) using the following PCR program: denaturation at 95°C for 5 min, followed by 24 cycles each with 95°C for 30 sec, 60°C for 90 sec and 72°C for 30 sec, followed by a final elongation step of 30 min at 60°C. The PCR reaction mix of 11.5 µL contained 6 µL of Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany), 0.2 µl of each 10 µM primer (10 µM), 1.5 µl of template DNA of unknown concentration, and 2.4 µl double-distilled water. The products of the two PCR reactions were purified separately using Sephadex (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), mixed 1:1 and separated on a capillary sequencer ABI 3130xl (Applied Biosystems) using GeneScan 600 LIZ® (Thermo Fischer Scientific) as internal size standard following the manufacturer's instructions.

Fragments were sized and manually scored using GeneScan 3.7 and Genotyper 3.7 (both Applied Biosystems). Assignment of alleles of equally labelled loci (e.g. Na12-C08 and Na12-E01) was based on information on allele size range available from previous studies (Pascher et al. 2000, 2006). For primer pair Na10-C01, no literature values for scoring were available. As this primer consistently amplified two distinct loci, that showed independent patterns of variation, both were scored (henceforth listed as Na10-C01a and Na10-C01b) and included in the matrix, which eventually contained 2,185 samples. Replicates resulted, as expected, in profiles congruent with those of the original samples - four replicates failed to amplify completely or nearly so - and were removed, resulting in a matrix of 2,116 samples.

This raw data matrix was further processed as follows. Samples that did not amplify for more than one locus (not considering Na12-E01b; see Chapter Results) even after the second attempt were removed (38 samples), reducing the data matrix to 2,078 samples. Alleles which did not fit into the expected step-size range (all amplified loci have two-base pair motifs: Lowe et al. 2004) were re-coded as missing data: one each in both loci of Na10-C01; two each in Na12-C06, Na12-E01a and

Na12-C08 and 13 in Na12-E06a (21 alleles in 20 samples). Likewise, loci with more than two alleles were coded as missing data, ignoring alleles that in the previous step had already been coded as missing data (one case each for loci Na12-E01a, Na12-C08 [in the same individual], and Na12-E06a), affecting five times Na12-A08, ten times locus Na12-C08 and 15 times locus Na12-E06a. Three samples that after this re-coding had two loci (again not considering Na12-E01b) with missing data were removed, resulting in a final matrix containing 2,075 samples.

7.7. Statistical analyses – microsatellite data-set

Data descriptors and diversity statistics (both for loci, populations, and population groups) were calculated using GenAlEx 6.5 (Peakall & Smouse 2006, 2012) and - for allelic richness only - Fstat 2.9.3.2 (Goudet 1995, 2001). As these programs cannot handle mixtures of missing data and allele sizes within the same locus of a sample, in four cases (one each in Na12-E01a and Na12-C08 and twice in Na12-C06) the observed allele was re-coded as missing data. Diversity measures were compared using Mann-Whitney tests or Kruskal-Wallis tests using PAST 3.09 (Hammer et al. 2001), where p-values were estimated using 9,999 Monte Carlo permutations. Differentiation among seven pre-defined population groups (BH: railway stations; FB: switchyards; GBH: border railway stations; DH: ports; S: road sections; OEM: oil mills; commercial varieties) was quantified using both a two-level AMOVA, where population groups are treated as populations, and a three-level AMOVA (populations with fewer than ten individuals were removed: BHK1, BHK4, BHK6, BHR10, GBH3, GBH7, OEM2, SR09) in Arlequin 3.5 (Excoffier & Lischer 2010); significance of F_{ST} values was estimated using 1,000 permutations. Population pairwise F_{ST}s, using number of different alleles as distance method (Weir & Cockerham 1984; Michalakis & Excoffier 1996), were calculated using Arlequin 3.5; significance of F_{ST} values was assessed using 1,000 permutations. Allelic richness accumulation curves were estimated for each population group using the R-package ARES 1.2-2 (van Loon et al. 2007) run on R 2.4.1 for Windows (R Development Core Team 2008, available from: https://cran.r-project.org/) with a maximum of 1,000 individuals (thus, at least twice the size of a given population group) and 500 bootstrap replicates to obtain confidence intervals. As marker Na12-E01b had 51.57% missing data (see results), all estimates were calculated both using the entire data set of ten loci and a reduced data set of nine loci (i.e., without Na12-E01b). Arlequin by default removes loci with more than 5% missing data. Consequently, calculations of AMOVA and pairwise F_{ST}s are based on the reduced data set.

Population structure was inferred using Structure 2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007) assuming admixture and correlated allele frequencies. The admixture model allows that individuals may have (but do not need to have) mixed ancestry; the correlated allele frequency model allows allele frequencies between inferred populations to be quite similar, which tends to improve clustering for closely related populations (Falush et al. 2003). For each number of clusters *(K)*, ranging from *K*=1 to *K*=20, ten independent runs were performed using a burn-in of 2×10^5 iterations followed by 2×10^6 additional MCMC iterations for sampling. For detecting the number of clusters, we used the DeltaK statistic of Evanno et al. (2005), calculated using StructureHarvester Web 0.6.94 (Earl & von Holdt 2012). The cluster output from Structure was aligned using Clumpp 1.1.1 (Jakobsson & Rosenberg 2007) and visualized using Distruct 1.1 (Rosenberg 2004). For comparative purposes, we additionally inferred population structure using BAPS 6 (Corander et al. 2003, 2004) with fixed *K* (calculations treating *K* as random variable, the default option in BAPS, resulted in unreasonably high estimates of *K* of larger than 50; data not shown). Each run was replicated ten times and the results were averaged according to the resultant likelihood scores. Admixture coefficients of individuals were estimated using 500 iterations, and the significance of these coefficients was estimated by employing the simulation strategy described by Corander & Marttinen (2006) using 50 reference individuals and 100 iterations each.

7.8. Statististical analysis – fielddata (data base)

All data registered during fieldwork were entered into an Access 2010 database. Statistical analysis was performed using R 3.2.2 (R Development Core Team 2008). Differences in the presence of feral OSR populations within or outside OSR cultivation areas in Austria were tested with Wilcoxon's rank test. Furthermore, for multiple comparisons of transport categories Tukey's HSD test was used. ANOVA and Generalised Linear Models were applied to test, whether OSR cultivation areas and transport categories would influence the population sizes/individual number of feral OSR plants in the sample sites.

8. Varieties of oilseed rape

8.1. Information on OSR varieties and organisation of seed samples

In total, we received 46 OSR varieties which were grown during the last ten years as far as they were still available from three of the four breeders and additionally, the two OSR varieties Honk and Mohican provided by H. Reiner (Table 12). Because of confidential reasons we were not able to get OSR varieties from Pioneer Hi Bred Services GmbH which has an OSR market share of only 1-3%. Moreover, sampling the small Oil Mill Raab which produces organic OSR oil, we received an organic variety seed sample of Romania. The name of the variety was unknown to the oil mill manager who imported organic OSR from Romania in 2014. The seed sample may be a bulk mixture of different organic OSR varieties, all complying with the same quality standards concerning content of oil and freedom of glucosinolates and erucic acid. Unfortunately, also this seed mixture did not germinate at all. We also got a seed sample from the warehouse in Marchegg as well as from the Oil Mill Fandler. Hence, altogether 51 OSR variety seed samples were germinated for analyses (Table 12), 48 of those are known varieties.

We received detailed information from the Saatzucht Donau GesmbH (Johann BIRSCHITZKY) concerning the provided OSR variety seed samples. In case of OSR, the main cultivated varieties change frequently that means a special OSR variety is cultivated only during a few years in succession and is then taken from the market. At present, no OSR variety which was listed in the Austrian plant variety data base 2005, the so called Österreichische Sortenliste 2005, is cultivated anymore. From the varieties used between 2004 and 2010/12 and which had a certain importance we received the varieties Californium, Caracas, Carousel, Mohican, and Tenno. Some varieties such as Honk or Columbus had importance fifteen to twenty years ago but not in the last ten years. No seed samples were available from the requested OSR varieties Artus, Baldur, and Express because they are old varieties and completely withdrawn from the market (pers. communication Dr. Blaimauer, RWA). In Austria as well as in several other countries, the importance of hybrid OSR varieties has increased intensely during the preceding years and currently has a market share of 76%.

In the Austrian plant variety data base 2010 (Österreichische Sortenliste 2010) a division between "variety lines" ("Liniensorten") synonymous with the term "open pollinators" used in Table 13 and hybrid varieties is made. There are several varieties which were already admitted in 2005, others still have importance for present cultivation. The provided varieties Ladoga, Henry, and NK Petrol had some importance in the last five to seven years, but are not on the market anymore.

Varieties from abroad:
We received six line varieties (Ricky, Tommy, Jimmy, Lenny, Freddy, Jolly) and the 2 hybrids (Orlando, Pedro) which are registered in the neighbouring countries of Austria. The mentioned OSR varieties are also listed in the EU plant variety data base ("EU Sortenliste") which can be found under the link http://ec.europa.eu/food/plant/propagation/catalogues/database/public/ (see Table 10).
The "plant variety data base" of the respective country can be seen on the homepages of the offices for varieties of the respective country: Hungary – NEBIH, Slovakia – UKSUP, Czech Republic – UKSUZ, Poland – COBORU, Romania – ISTIS, Germany – BSA. For example, a selection of the variety database of Hungary from NEBIH is given in Table 11 (last data from 2007, https://www.nebih.gov.hu/en/specialities/inst_agro/ag_data/natinv.html).

Table 10: Analysed OSR varieties from abroad provided by the Saatzucht Donau. Data from the EU plant variety data base, agricultural plant species (A-61 Swede rape). http://ec.europa.eu/food/plant/propagation/catalogues/database/public/.

OSR variety	Common catalogue status: registration	Country of admission	National list status: admission/deletion date
FREDDY	Registered	Hungary (HU)	admission: 17.11.2009
JIMMY	Registered	Croatia (HR)	admission: 31.08.2010
	Registered	Hungary (HU)	admission: 17.11.2009
	Registered	Slovacia (SK)	admission: 23.03.2010
JOLLY	Registered	Hungary (HU)	admission: 10.03.2011
LENNY	Registered	Denmark (DK)	admission: 14.02.2012
	Registered	Hungary (HU)	admission: 10.03.2011
	Registered	Slovacia (SK)	admission: 04.04.2012
ORLANDO	Deleted	United Kingdom (UK)	admission: 04.02.2000
			deletion: 30.04.2004
	Registered	Croatia (HR)	admission: 15.06.2012
	Registered	Italy (IT)	admission: 29.06.2012
PEDRO	Registered	Croatia (HR)	admission: 13.03.2012
RICKY	Registered	Hungary (HU)	admission: 08.12.2011
TOMMY	Registered	Croatia (HR)	admission: 04.08.2009
	Registered	Hungary (HU)	admission: 14.11.2007

Table 11: Variety database of Hungary from NEBIH.
(https://www.nebih.gov.hu/en/specialities/inst_agro/ag_data/natinv.html)

Nicode	Instcode	Accenumb	Genus	Species	Subtaxa	Cropname	Acqdate	Origcty
HUN	HUN003	RCAT015957	*Brassica*	*Napus*	subsp. *napus*	Valocevska	1972----	
HUN	HUN003	RCAT015958	*Brassica*	*Napus*	subsp. *napus*	Uj Fertôdi	1973----	HUN
HUN	HUN003	RCAT015959	*Brassica*	*Napus*	subsp. *napus*	Gorczanski	1979----	POL
HUN	HUN003	RCAT015960	*Brassica*	*Napus*	subsp. *napus*	IR-1 fj	1976----	HUN
HUN	HUN003	RCAT015961	*Brassica*	*Napus*	subsp. *napus*	Rapora	1976----	DEU
HUN	HUN003	RCAT015962	*Brassica*	*napus*	subsp. *napus*	Primor	1976----	FRA
HUN	HUN003	RCAT015963	*Brassica*	*napus*	subsp. *napus*	Erra	1978----	DEU
HUN	HUN003	RCAT015964	*Brassica*	*napus*	subsp. *napus*	Girita	1978----	
HUN	HUN003	RCAT015965	*Brassica*	*napus*	subsp. *napus*	IR-022	1978----	HUN
HUN	HUN003	RCAT015966	*Brassica*	*napus*	subsp. *napus*	Solux	1978----	DDR
HUN	HUN003	RCAT015967	*Brassica*	*napus*	subsp. *napus*	GK. Savaria	1978----	HUN
HUN	HUN003	RCAT015968	*Brassica*	*napus*	subsp. *napus*	No.2	1978----	CAN
HUN	HUN003	RCAT015969	*Brassica*	*napus*	subsp. *napus*	SV 74/19	1978----	SWE
HUN	HUN003	RCAT015970	*Brassica*	*napus*	subsp. *napus*	SV 749465	1978----	SWE
HUN	HUN003	RCAT015971	*Brassica*	*napus*	subsp. *napus*	GK. Terra	1979----	HUN
HUN	HUN003	RCAT015972	*Brassica*	*napus*	subsp. *napus*	Quinta	1979----	DEU
HUN	HUN003	RCAT015973	*Brassica*	*napus*	subsp. *napus*	No.1	1976----	CAN
HUN	HUN003	RCAT015975	*Brassica*	*napus*	subsp. *napus*	No.3	1978----	CAN

Table 12: OSR varieties grown in Austria during the last ten years and selected available OSR varieties imported from EU countries to Austria. OSR variety seed samples were requested from the Saatzucht Donau GesmbH & CoKG, the Raiffeisenware Austria (RWA AG) and from the KWS Austria Saat GmbH. Due to concern of confidentiality no seed samples from Pioneer Hi Bred Services GmbH were received.
Abbreviations: CPB…former name of KWS UK Ltd., SZD…Saatzucht Donau, NPZ…Norddeutsche Pflanzenzucht, HY…hybrid, OP…open pollinators (= variety lines), BSL…Österreichische Beschreibende Sortenliste.

Name of variety	Applicant in Austria	Breeder	Seed after harvest	Type	List of varieties
Adriana	RWA	F	2013	OP	BSL
Alabaster	Saatzucht Donau	Limagrain	2013	HY	BSL
Albatros	Saatzucht Donau	Limagrain, F	2013	HY	BSL
Ametyst	RWA	S	2013	OP	BSL
Artoga	Saatzucht Donau	Limagrain, F	2013	HY	BSL
Californium	Saatzucht Donau	Monsanto	2006 ?	OP	BSL
Caracas	Saatzucht Donau	Monsanto	2005 ?	OP	BSL
Carousel	Saatzucht Donau	Monsanto	2002 ?	OP	BSL
Casoar	Saatzucht Donau	Monsanto, USA	2013	OP	BSL
Castille	Saatzucht Donau	Monsanto, USA	2005 ?	OP	BSL
Columbus	Saatzucht Donau	Monsanto	2002 ?	OP	BSL
Contact	Saatzucht Donau	Monsanto	2005	OP	BSL
Digger	KWS				BSL
DK Excellium	Saatzucht Donau	Monsanto, USA	2013	HY	BSL
DK Exfield	RWA	Monsanto, USA	2013	HY	BSL
DK Expertise	Saatzucht Donau	Monsanto, USA	2013	HY	BSL
DK Expower	Saatzucht Donau	Monsanto, USA	2013	HY	BSL
DK Exstorm	RWA	Monsanto, USA	2013		BSL
DK Sequoia	RWA	Monsanto, USA	2013		BSL
ES Solist	RWA		2013		BSL
Gloria	Saatzucht Donau	Syngenta, CH	2013	OP	BSL
Graf	Saatzucht Donau	Monsanto, USA	2013	HY	BSL
Harry	Saatzucht Donau	SZD, A	2010	OP	BSL
Henry	Saatzucht Donau	SZD, A	2013	OP	BSL
Honk	Saatzucht Donau	Groenbroek	???	OP	BSL
Kutiba	RWA		2011		BSL
Landoga	Saatzucht Donau	Limagrain	2013	OP	BSL
Mickey	Saatzucht Donau	SZD, A	2007	OP	BSL
Mohican	Saatzucht Donau	CPB	???	OP	BSL
NK Petrol	Saatzucht Donau	Syngenta	2013	HY	BSL
Peter 29	RWA		2013		BSL
Remy	KWS	D		OP	BSL
Sammy	Saatzucht Donau	SZD, A	2008	OP	BSL
Sherlock	KWS	D		OP	BSL
Sherpa	RWA	D	2013		BSL
Sidney	Saatzucht Donau	SZD	2011	OP	BSL
Tenno	Saatzucht Donau	NPZ	2006 ?	HY	BSL
Viking	Saatzucht Donau	NPZ	2002 ?	HY	BSL
WKR Janus	RWA		2012		BSL
DK Sedona	Saatzucht Donau	Monsanto	2010	HY	EU
Freddy	Saatzucht Donau	SZD	2013	OP	EU
Jimmy	Saatzucht Donau	SZD	2007	OP	EU
Jolly	Saatzucht Donau	SZD	2011	OP	EU
Lenny	Saatzucht Donau	SZD	2011	OP	EU
Orlando	Saatzucht Donau	Monsanto/SZD	2008	HY	EU
Pedro	Saatzucht Donau	Monsanto/SZD	2008	HY	EU
Ricky	Saatzucht Donau	SZD	2013	OP	EU
Tommy	Saatzucht Donau	SZD	2012	OP	EU
Marchegg: warehouse	?	?	?	?	
Fandler Oil Mill	?	?	?		
Raab Oil Mill	Organic: Romania	?	2014	?	EU?
			Import		

8.2. Most frequently cultivated OSR varieties in Austria

According to the information from the AGES (Dr. H.W. LUFTENSTEINER, Department: Testing and Evaluating of Crop Varieties) the best sources to assess the relevance of specific OSR varieties for the domestic OSR cultivation in Austria are the Vereinigung der Saatgutkaufleute ("Association of Seed Traders"), for instance the RWA, Saatbau Linz and the KWS. Assessment should be possible based on sales figures or amounts. This would be true for import of OSR seed as well as consumption ware. Unfortunately, it was not possible to get access to data on market shares of OSR varieties. Another potential data source for further information about the relevance of single OSR varieties for cultivation, amount and local use could be the so called Austrian "Landwirtschaftskammern" (https://www.lko.at/, especially the ones of Lower Austria, Upper Austria and Burgenland). However, for the project at hands this suggestion could not be implemented.

Hence, the importance of certain varieties was concluded based on information which was available publicly (e.g. Österreichische beschreibende Sortenliste 2014, personal information). The variety California had high importance in the years of harvest around 2005 and 2006. Also important varieties in these years were Baldur (Hybid: Hyb), Explus (Hyb), Caracas, and Siska with variable cultivation proportion. Then Baldur started to be cultivated more frequently. Also the hybrid Exagone was of large account. Casoar and Pulsar (Hyb) were also relevant varieties at that time. Popular varieties during the following years were Visby (Hyb), Artoga (Hyb), Exstorm (Hyb), Sherpa (Hyb), and Explicit (Hyb). Even only rough data were available, it is clearly visible that hybrid varieties have started to account more and more during the last years. Also DK Exssence (Hyb), DK Explicit (Hyb), Arsenal (Hyb), and DK Exstorm (Hyb) came on the market and were frequently used. As mentioned above, at present OSR hybrid varieties have a market share of 76%. The following information (see Table 13) refers to the region Bruck/Schwadorf.

Table 13: Importance of certain OSR varieties grown in Austria in the region of Bruck/Schwadorf. Estimations provided by Ing. Michael Kober and his colleague from RLG Bruck. According to their statement it only serves as an orientation.

OSR variety	Dominance	Dominance within years	Warehouse amount in %	OSR variety	Dominance	Dominance within years	Warehouse amount in %
Adriana	Yes	2012 - 2015	10%	Harry	no		
Alabaster	No			Henry	no		
Albatros	No			Honk	no		
Ametyst	No			Kutiba	no		
Artoga	Yes	2015 - 2015		Landoga	no		
Californium	Yes	2009 - 2015	10%	Mickey	no		
Caracas	No			Mohican	no		
Carousel	No			NK Petrol	yes	2009 - 2011	5%
Casoar	Yes	2010 - 2015	5%	Peter 29	no		
Castille	No			Remy	yes	2007 - 2011	10%
Columbus	Yes	ca. 2000 - 2008	2%	Sammy	no		
Contact	No			Sherlock	yes	2011 - 2014	5%
Digger	No			Sherpa	yes	2011 - 2014	5%
DK Excellium	No			Sidney	no		
DK Exfield	No			Tenno	no		
DK Expertise	No			Viking	no		
DK Expower	No		5%	WKR Janus	no		
DK Exstorm	Yes	2011 - 2015	20%				
DK Sequoia	No			Visby	yes	2009 - 2014	15%
ES Solist	No			Artus	yes	2003 - 2007	25%
Gloria	No			Express	yes	1999 - 2005	25%
Graf	Yes	2014 - 2015	5%	Pulsar	yes	ca. 2010 - 2014	25%

No seed was imported from abroad in that region. At the beginning of the OSR production the varieties Express, Artus, and Pulsar had a considerable share of cultivation areas. At the beginning of intense OSR cultivation in Austria, the hybrid Artus and the line variety Express were dominant. Due to the increased diversity of OSR varieties the dominance of single varieties declined. The situation today is that OSR cultivation is distributed among several varieties per seed producer. This information matches with the experience in our studies. For the time-period between 1990 and 1999 we received only 13 OSR varieties for the study (PASCHER et al. 2000, 2010). In comparison, in the present study 40 OSR varieties cultivated in Austria during the last ten years (2005-2014) and 8 EU varieties could be organised which, however, does not present the complete variety set of those years.

Only the Oil Mill Rapsöl Braunshofer responded to requests to indicate which varieties had been processed in the mill in 2014: Visby, Casoar, and Artoga with 75% regional cultivation and pioneer hybrid (pers. Information Aleksandra Braunshofer). Most of the oil mills received OSR seeds as bulk mixture complying with quality standards, but without certain variety information.

8.3. Breeding and characteristics of OSR varieties

A hybrid variety is produced from two parental lines with different genetic backgrounds each. The F1 generation is genetically uniform and because of the heterosis-effect the F1 surpasses each of their parental varieties in agronomical relevant traits. However, this effect is only seen in the first generation. Due to the Mendelian segregation of parental alleles, the heterosis-effect is lost or diminished in subsequent generations. Single genes of the F2 generation split into 25% equal to line A (first parent), 50% are heterozygous (hybrids) and 25% are equal to those of line B (second parent).

Variety purity especially of the crop OSR is in general very hard to be achieved by the breeders because of the typical characteristics of OSR such as a high potential for hybridisation and admixture, a fact to be considered in our study. Feral OSR plants which trace back to a hybrid variety can also be found in the F2 generation in three varieties: line A, line B or hybrids (heterozygous). Only the parental OSR variety lines are genetically uniform. At present, more than 75% of the used OSR varieties are hybrids. According to the statement of Dr. Blaimauer (RWA), one has to accept this special circumstance of OSR which makes genetic testing of OSR in comparison to other crops such as maize especially difficult. Moreover, he predicted that due to this complication we would have to expect and accept that definite assignment to OSR varieties will hardly be possible in all cases.

For reasons of identification and traceability the names of varieties produced by a certain plant breeding company start with the same letter. For example, the L-varieties go back to the German “Saatveredelung in Lippstadt“. If there is an initial of DK in the name of a variety, it is produced by Monsanto. The company Monsanto does not only produce GM varieties, but also many other conventional ones. The company also develops GM varieties which serve for differing marketing strategies. DeKalb is a company in Düsseldorf also belonging to Monsanto - 1. big US seed company. Artus was one of the most relevant hybrid varieties in Austria.

In general, varieties coming from Hungary and Slovakia have the largest relevance for Austria.

In 1985/86, only 2,000 to 4,000 ha were cultivated with OSR which still contained erucic acid and glucosinolates. Since 1988 and 1989, “00”-varieties of OSR have been on the market. They are free of erucic acid and glucosinolates. Byproducts produced from “00”-OSR varieties such as OSR grist and press cake (“Presskuchen”) can be used as animal feedstock.

8.4. Identification of specific OSR varieties

There was no information available concerning single imported OSR varieties. Statistik Austria could just provide information from which countries and in which amount oil seed is imported. As mentioned above, data concerning import of oil seed are not split into separate categories such as OSR. The information included the amount in kg, value in Euro and the importing country. Equally, also from the oil processing facilities we did not get any information about purchased OSR varieties. Also the IACS-data do not make variety specific information in the farmers' Austrian cultivation data available. Concerning this topic, we also did not receive detailed data from the AGES.

From the Rapso-Mühle in Aschach (Herr WILDLING, personal communication) we got the detailed information that 199,000 t OSR were imported in a crushed form to the oil mill and in average in total 330,000 t are processed to edible oil. According to the received information, small amounts of OSR are imported to decentralised oil mills and processed to biofuel which is used as fuel for the machinery operated by these businesses ("Maschinenring"). At present, OSR does not generally play a major role as feedstock for production of biofuels in Austria (MOSER et al. 2013).

9. Results

9.1. Occurrence of feral oilseed rape plants

Figure 23 shows the location of the selected 60 sample sites at which the feral OSR plants were sampled during field work in 2014 and 2015. Feral OSR was present on 44 of the 60 investigated sites during the two sampling years. Most of these locations (ports, railway stations, switchyards, border railway stations, transportation roads, oil mills, and processing company) are situated in the Austrian OSR cultivation areas (marked in light yellow). On 16 sites no feral OSR plant was observed in both years. The type of sample sites, the location name, the Federal States in which they are situated as well as the number of sampled individuals are listed in Table 14. It is obvious that with a few exceptions no feral OSR plant could be found in the sampled road sections along roads (e.g. Salzburg) which lead through Austrian areas without OSR cultivation and probably without OSR transportation.

For each sample site a comprehensive photographic documentation was made. Figure 22 shows a border railway station (GBH1 Nickelsdorf) with masses of OSR plants occurring along the railway lines.

Figure 22: Yellow strips of feral OSR along railway line (GBH1: Nickelsdorf).

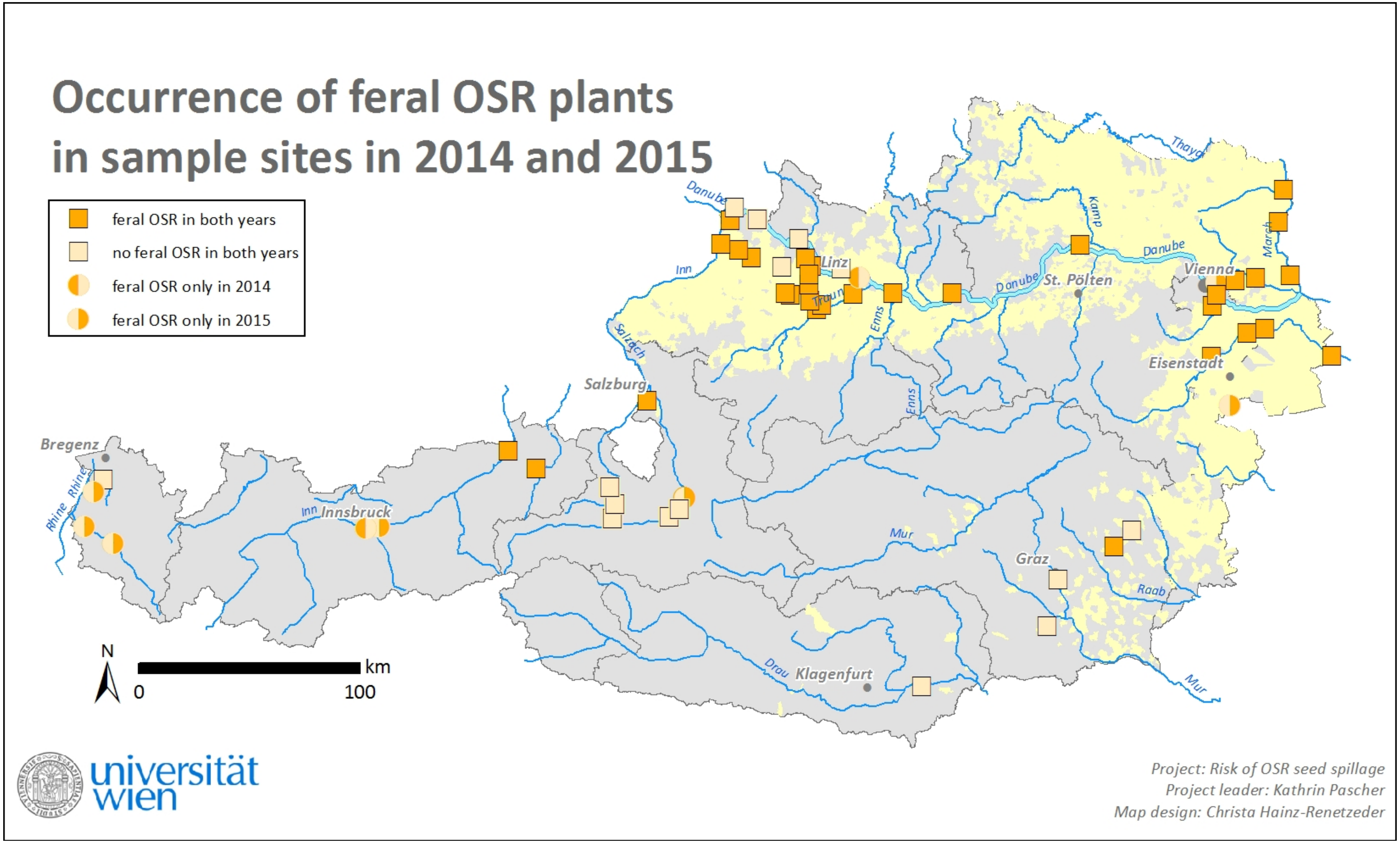


Figure 23: Location of the 60 sample sites. Registration of feral OSR in the sample sites in 2014 and/or 2015.

Table 14: Sample sites and number of sampled feral OSR plants.

Sampling type	Sample sites	Federal State	Sampled ind.	Sampled ind.
Railway stations within (BHR) Austrian OSR cultivation areas			2014	2015
BHR1	Wels - Main Station	Upper Austria	26	47
BHR2	Schlüsslberg	Upper Austria	11	3
BHR3	Raasdorf	Lower Austria	5	18
BHR4	Siebenbrunn-Leopoldsdorf	Lower Austria	26	29
BHR5	Grieskirchen	Upper Austria	21	28
BHR6	Trautmannsdorf a. d. Leitha	Burgenland	17	11
BHR7	Wampersdorf	Lower Austria	1	10
BHR8	Traun	Upper Austria	1	25
BHR9	Dürnkrut	Lower Austria	53	38
BHR10	Hirschstetten-Aspern	Lower Austria	0	1
Railway stations outside (BHK) Austrian OSR cultivation areas				
BHK1	Völs	Tyrol	3	0
BHK2	Linz – Wahringerbahnhof	Upper Austria	40	0
BHK3	Innsbruck – Main Station	Tyrol	0	28
BHK4	Hohenems	Vorarlberg	0	1
BHK5	Graz - Railway Station East	Styria	0	0
BHK6	Frastanz	Vorarlberg	0	1
BHK7	Preding-Wieselsdorf	Styria	0	0
BHK8	Bludenz	Vorarlberg	0	11
BHK9	Dornbirn	Vorarlberg	0	0
BHK10	Völkermarkt-Kühnsdorf	Carinthia	0	0
Railway stations on Austrian borders				
GBH1	Nickelsdorf	Lower Austria	24	50
GBH2	Hohenau	Lower Austria	50	49
GBH3	Baumgarten	Burgenland	0	2
GBH4	Kufstein	Tyrol	2	18
GBH5	Marchegg	Lower Austria	40	43
GBH7	Salzburg	Salzburg	1	2
Switchyards				
FB1	Kledering	Lower Austria	98	60
FB2	Wels	Upper Austria	21	47
Ports				
DH1	Port Albern	Vienna/Lower A.	35	61
DH2	Port Krems	Lower Austria	55	64
DH3	Port Enns	Upper Austria	30	50
DH4	Grein	Upper Austria	20	22
DH5	Obermühl an der Donau	Upper Austria	0	0
DH6	Wilhering	Upper Austria	0	0

Table 14 continued

Sampling type	Sample sites	Federal State	Sampled ind.	Sampled ind.
Road sections	within the Austrian OSR cultivation area		2014	2015
SR01	Katzdorf bei Wallern a. d. Trattnach	Upper Austria	30	34
SR02	Aumühle bei Wels	Upper Austria	30	50
SR03	Thall bei Waizenkirchen	Upper Austria	0	0
SR04	Taufkirchen- Leoprechting	Upper Austria	20	40
SR05	Thalmannsbach	Upper Austria	20	40
SR06	St. Florian am Inn	Upper Austria	12	15
SR07	Hinding (an der Donau)	Upper Austria	0	0
SR08	Schardenberg-Steinbrunn	Upper Austria	2	15
SR09	Pischelsdorf in der Steiermark	Styria	3	1
SR10	Dienersdorf-Kaindorf	Styria	0	0
SR11	Bruck an der Leitha	Lower Austria	49	58
Road sections	outside of the Austrian OSR cultivation area			
SK01	Eferding	Upper Austria	22	1
SK02	Hilkering bei Aschach an der Donau	Upper Austria	8	45
SK03	Pupping und Karling bei Aschach	Upper Austria	26	3
SK04	Engelhartszell (an der Donau)	Upper Austria	0	0
SK05	St. Johann im Pongau	Salzburg	0	0
SK06	Bischofshofen	Salzburg	0	4
SK07	Zell am See	Salzburg	0	0
SK08	Maishofen	Salzburg	0	0
SK09	Schwarzach im Ponau	Salzburg	0	0
SK10	Saalfelden am Steinernen Meer	Salzburg	0	0
SK11	Going (beim Wilden Kaiser)	Tyrol	0	0
Oil mills				
OEM1	Vereinigte Fettwaren - Wels	Upper Austria	21	0
OEM2	Fandler - Pöllau	Styria	0	2
OEM3	Bunge - Bruck an der Leitha	Burgenland	165	60
OEM4	Raab - Fraham	Upper Austria	13	25
		Samples:	**1,001**	**1,112**

In total, 2,113 feral plant individuals were sampled in 2014 and 2015, additionally 2 individuals of *Brassica rapa* in Tyrol. Figure 24 shows the occurrence of feral plants summarized for 2014 and 2015 for each sample site. If only few individuals were present at one site, all of these – with the exception of plants which could not be collected because of safety reasons – were sampled. However, there were sites with up to 1,500 plants (in 2014) such as SR02. The map indicates that the big yellow flowers (>50 individuals) are all situated in the light yellow coloured OSR cultivation area. In Figure 25 occurrence of feral OSR is pictured along transportation network. Nearly all feral plants exhibited high vitality. They blossomed and had already developed viable seeds which are a prerequisite for the persistence of a population over subsequent years.

Feral OSR was often invoked to be just a pioneer plant. However, feral OSR plants are able to settle very different habitats, a quality which is illustrated in Figure 26. An amazing observation was made at the port of Albern, where an OSR plant grew at the embankment of the River Danube rooting directly in the water which indicates to OSR's wide ecological habitat-spectrum.

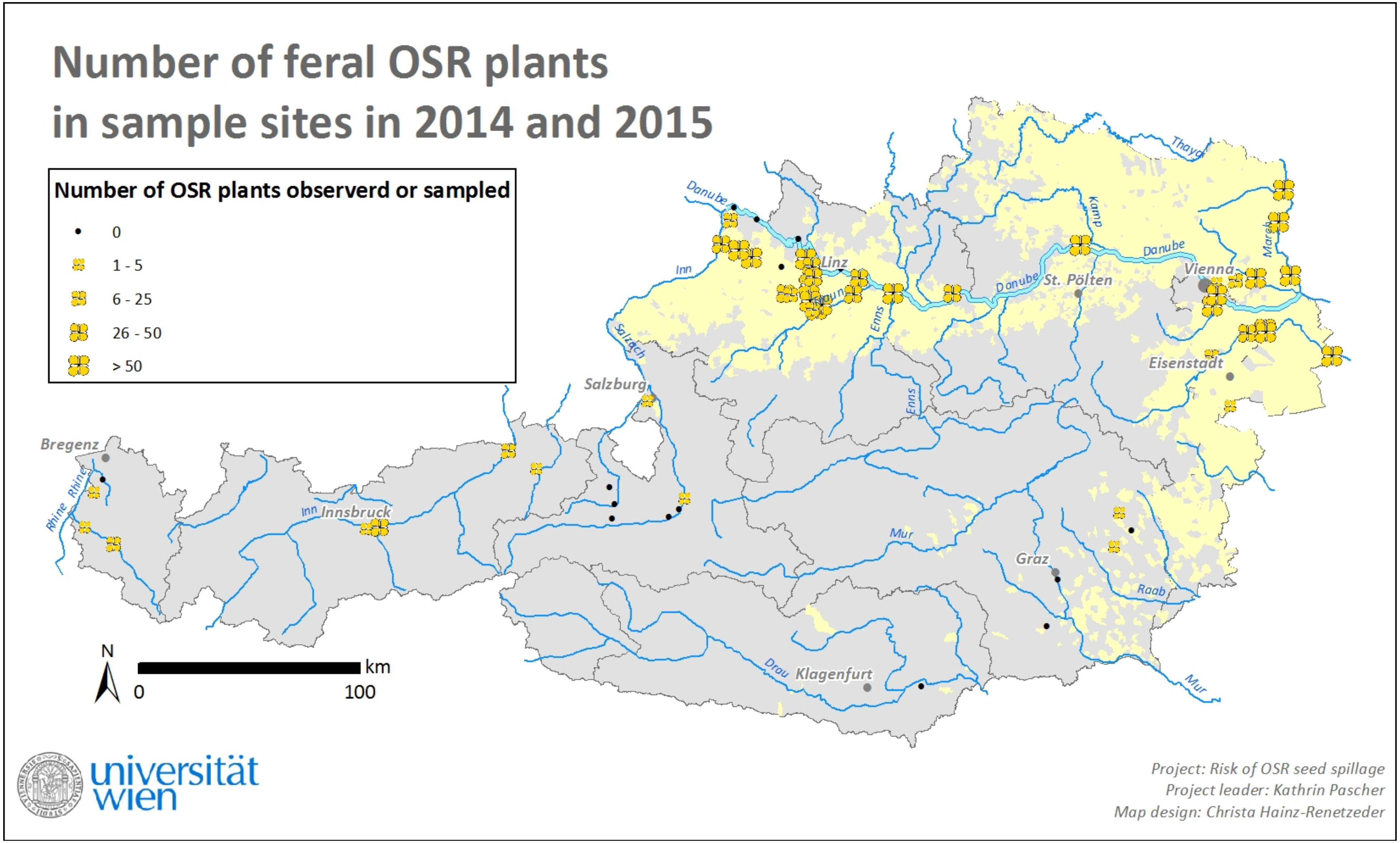


Figure 24: Occurrence of feral OSR plants in the sample sites summarized for both years, 2014 and 2015.

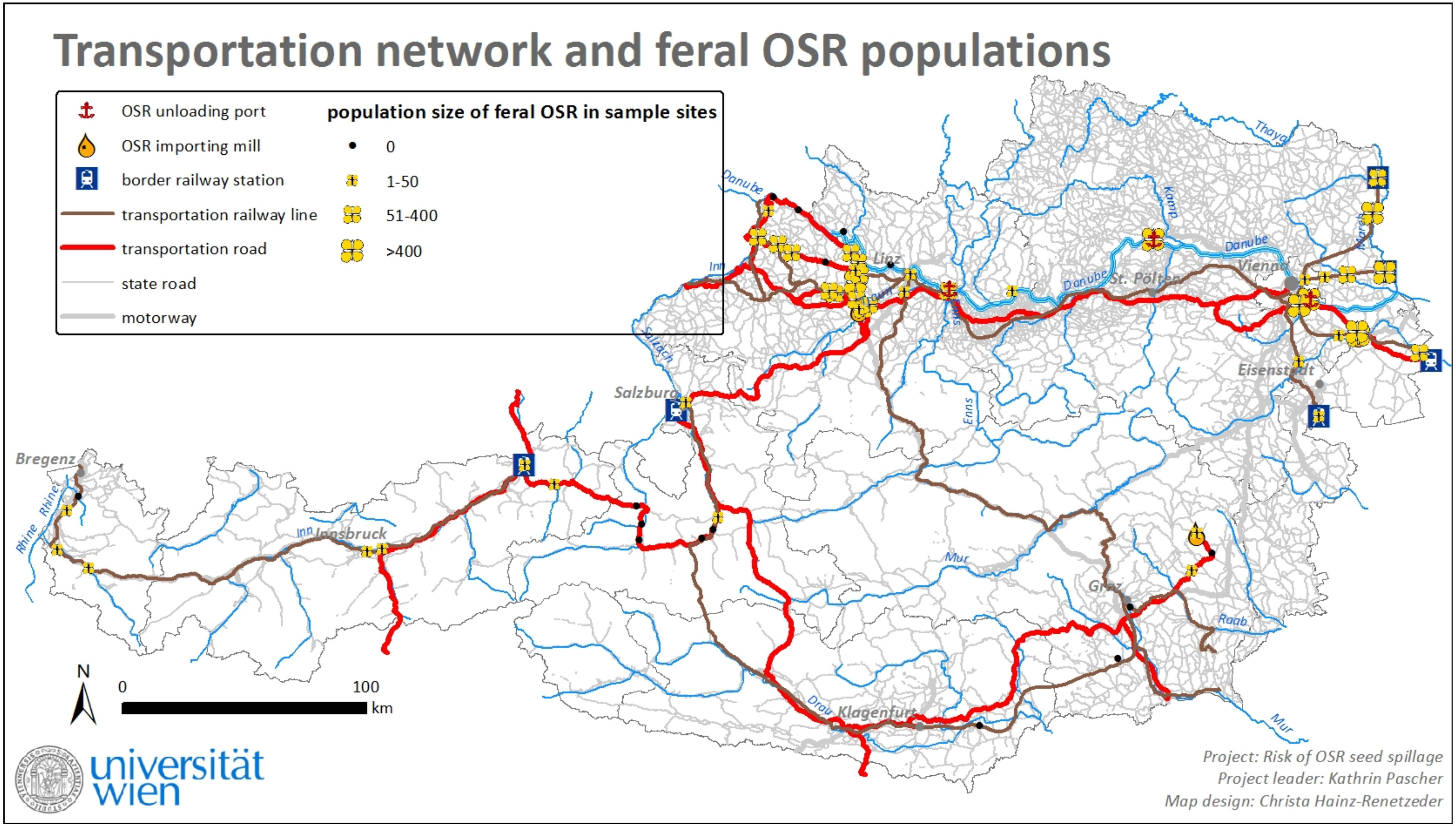


Figure 25: Occurrence of feral OSR plants in the sample sites along transportation network summarized for both years, 2014 and 2015.

Figure 26: Ecological habitat-spectrum ("Ökologische Standortsamplitude") of OSR. A selection from fieldwork at sample sites in 2014 and 2015.

9.2. Occurrence of hybridisation partners of OSR

Oilseed rape is able to hybridise with several closely related species (CHEVRÉ et al. 2004). PASCHER & GOLLMANN (1999) and PASCHER et al. (2000) identified more than 20 species as relevant hybridisation partners in Austria. Several of those species could be registered during field work in the 60 sample sites. They are listed in Table 15 and their occurrence is shown in Figure 27. The list of registered species may be incomplete because in some sample sites observation had to be focussed on OSR only due to safety reasons and limited observation possibilities. In total, 11 species of relevant hybridisation partners were registered in the sample sites with *Sinapis arvensis* and *Diplotaxis tenuifolia* as the two most common species (Table 15). In 25 sample sites, two species were registered, whereas only in one sample site up to five hybridisation partners were observed (Figure 28). Wilcoxon's rank test revealed no differences in the occurrence of hybridisation partners inside and outside OSR cultivation area (Figure 29).

Table 15: Relevant hybridisation partners of OSR registered in the 60 sample sites.

Species	Number of registered sample
Brassica napus (feral)	1
Brassica rapa (feral)	5
Diplotaxis tenuifolia	20
Erucastrum gallicum	4
Raphanus raphanistrum	7
Sinapis alba	17
Sinapis arvensis	21
Sisymbrium loeselii	15
Sisymbrium officinale	17
Sisymbrium orientale	3
Sisymbrium strictissimum	1

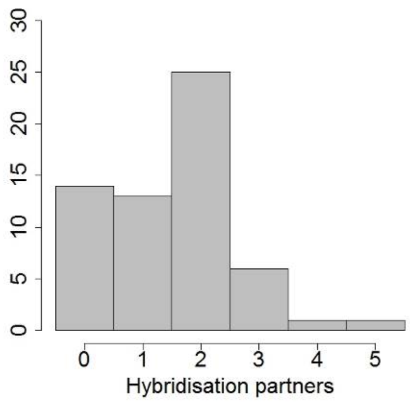


Figure 27: Frequency distribution of number of possible hybridisation partners of OSR in the sample sites.

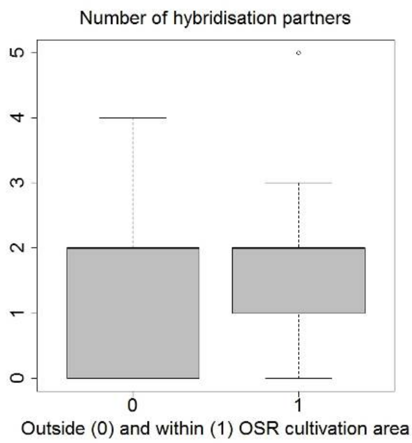


Figure 28: Number of hybridisation partners registered in the sample sites within and outside the OSR cultivation area.

Sinapis arvensis was the species most frequently found in 21 of altogether 60 sample sites (Figure 30). *Raphanus raphanistrum* is known for its contradirectional occurrence to *S. arvensis* and has a distribution hotspot in Upper Austria. Similar to the Austrian situation, also *Sinapis arvensis* was relatively common in the Swiss study (SCHULZE et al. 2014). *Diplotaxis tenuifolia* was the second frequently observed hybridisation partner (Figure 31). All of the plants flowered and produced seeds. This wild species closely related to OSR, is also very common in Switzerland. In the study of SCHULZE et al. (2014) it was preferentially found in embankments in high individual numbers.

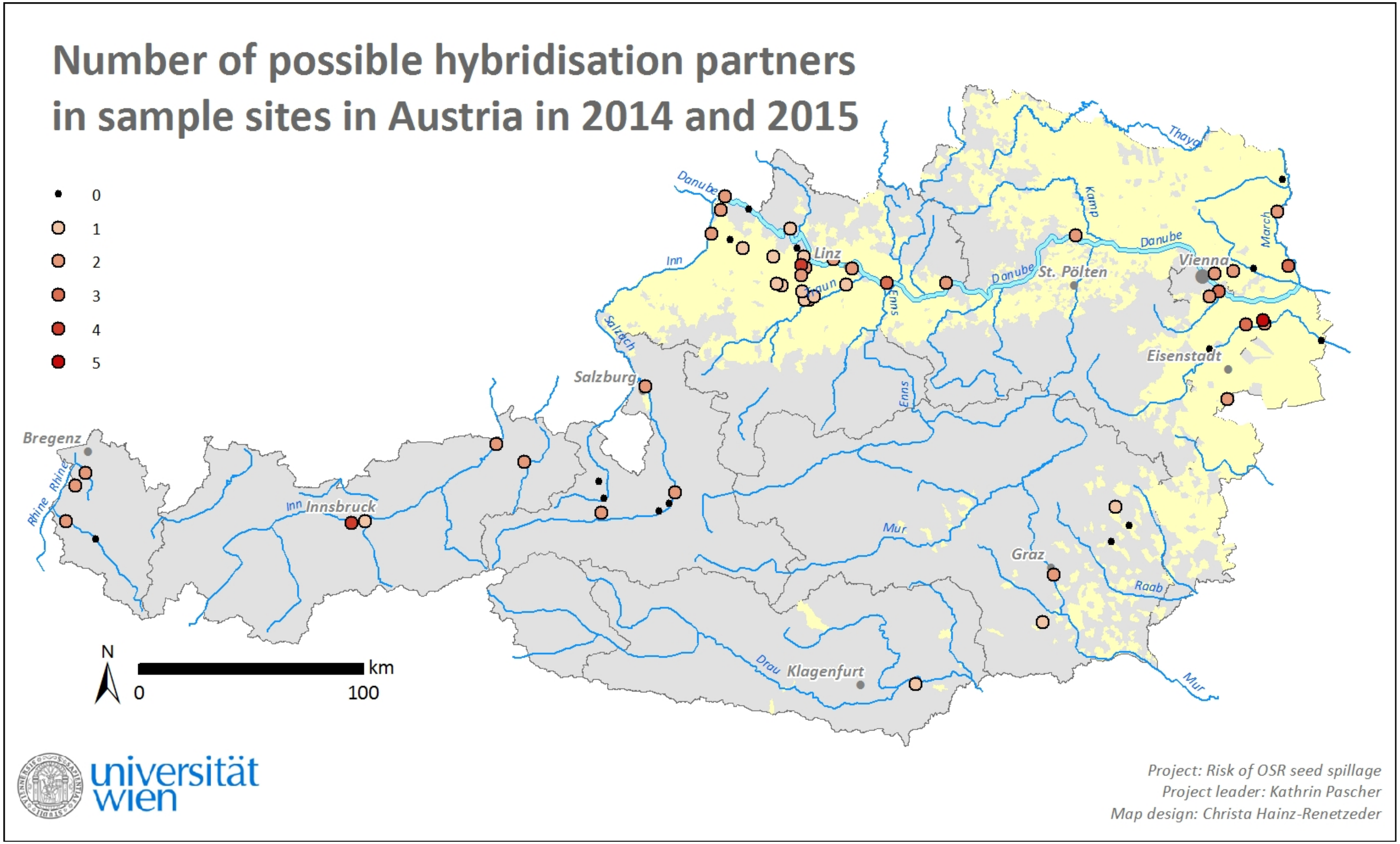


Figure 29: Number of observed Brassicaceae during sampling (the map does not claim to be exhaustive). The listed species were detected at the 60 sample sites: *Brassica rapa* (on 5 sites), *Diplotaxis tenuifolia* (20), *Erucastrum gallicum* (4), *Raphanus raphanistrum* (7), *Sinapis alba* (17), *S. arvensis* (21), *Sisymbrium loeselii* (15), *S. officinale* (17), *S. orientale* (3), and *S. strictissimum* (1).

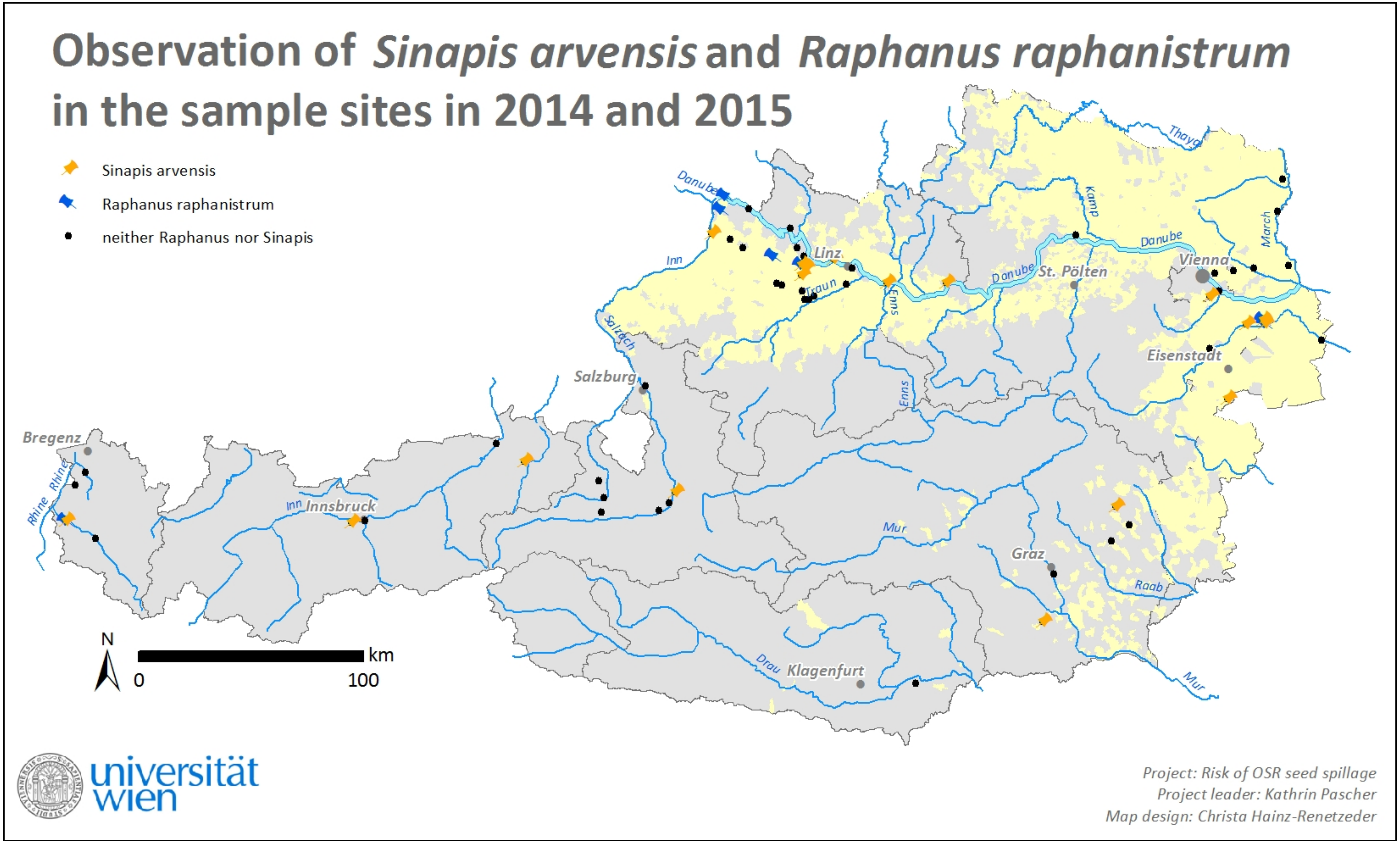


Figure 30: Records of *Sinapis arvensis* (yellow) and *Raphanus raphanistrum* (blue) in the sample sites in 2014 and 2015.

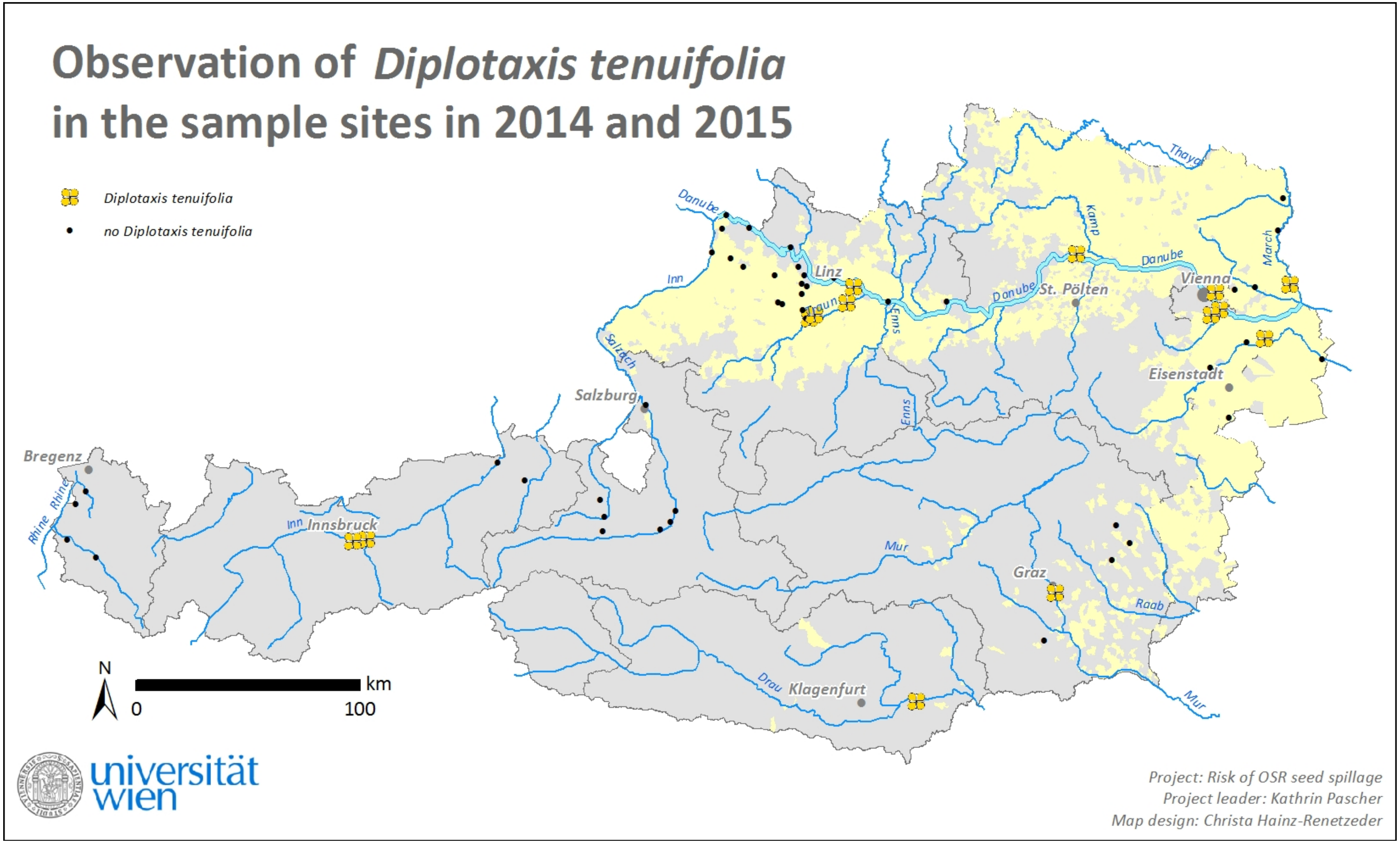


Figure 31: Records of *Diplotaxis tenuifolia* in the sample sites in 2014 and 2015.

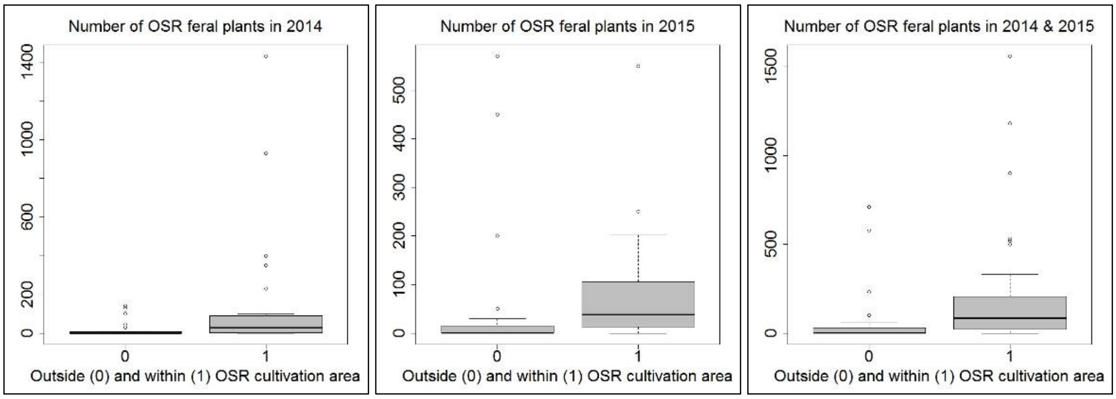
Apart from sampling size, population size of feral OSR plants at each sample-site was registered during field work. The population sizes show a large difference between the locations. There were sites with no feral OSR plant at all. Contrastingly, the absolute maximum with estimated more than 1,400 plants was found in sample site SR02 in Upper-Austria in 2014 (Table 16) and with several thousand plants at the oil mill Bunge. A median of 30 individuals was observed, summarized, for both years.

Table 16: Summary statistics of feral OSR occurrence in the sample sites.

	Individuals 2014	Individuals 2015	Individuals both years
Min.	0.00	0.00	
1st Qu.	0.00	0.00	0.75
Median	4.50	17.00	30.00
Mean	86.98	63.23	150.22
3rd Qu.	46.25	73.75	124.25
Max.	1430.00	570.00	1560.00

9.2.1. Differences within and outside OSR cultivation areas

Climatic conditions for OSR cultivation in Austria are described in Chapter 5: "Cultivation of oilseed rape (OSR) in Austria". Thus, the question arises whether feral OSR populations may be more present within this area or not. Is there a difference in the establishment of populations? Wilcoxon's rank test showed significant differences (see Figure 32).



a. p-value = 0.000969	b. p-value = 0.0009956	c. p-value = 0.0008896

Figure 32: Boxplots showing population sizes of feral OSR plants within and outside OSR cultivation areas. P-values resulted from Wilcoxon's rank test.

After adjusting data by removing outliers, an ANOVA was calculated (Table 17) confirming the trend recognized in the boxplot. Also the Generalised Linear Model (Table 18) attested a significant influence of the OSR cultivation area for the establishment of feral OSR populations. However, on some sample sites, large populations of feral OSR could establish in areas where no OSR is grown. There is a probability that import activities could have boosted those establishments, since local transport activities in the course of cultivation are not carried out here.

Table 17: Results of ANOVA for population sizes of feral OSR plants to test for significance of OSR cultivation area.

ANOVA Individuals for 2014 and 2015					
	Df	Sum	Sq Mean	Sq F value	Pr(>F)
	1	23697	23697	7.232	0.00976 **
Residuals	49	160559	3277		
ANOVA Individuals for 2014					
	Df	Sum	Sq Mean	Sq F value	Pr(>F)
	1	2712	2712.2	4.53	0.0384 *
Residuals	49	29335	598.7		
ANOVA Individuals for 2015					
	Df	Sum	Sq Mean	Sq F value	Pr(>F)
	1	10376	10376	5.192	0.0271 *
Residuals	49	97919	1998		

Table 18: Results of the Generalised Linear Models (GLM) for population sizes of feral OSR plants to test for significance of OSR cultivation area.

GLM (Indiv2014 ~ OSRarea, family = quasipoisson)	Min	1Q	Median	3Q	Max
Deviance Residuals	-17.179	-12.504	-5.958	-3.770	62.695
	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	2.8764	0.8737	3.292	0.0017 **	
OSR area1	2.1179	0.9185	2.306	0.0247 *	
GLM (Indiv2015 ~ OSRarea, family = quasipoisson)	Min	1Q	Median	3Q	Max
Deviance Residuals	-12.319	-9.878	-7.796	0.387	41.951
	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	3.8874	0.4349	8.938	1.68e-12 ***	
OSR area1	0.4416	0.5437	0.812	0.42	
GLM (Indiv_all ~ OSRarea, family = quasipoisson)	Min	1Q	Median	3Q	Max
Deviance Residuals	-21.139	-11.704	-10.623	-4.473	58.224
	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	4.1977	0.5311	7.904	8.89e-11 ***	
OSR area1	1.2114	0.5963	2.031	0.0468 *	

Differences between transport and handling systems

Tukey's HSD test compared multiple means and needs, thus to be regarded with care since our data have no Gaussian Distribution, and sample sizes differ from each other to a high extent (Figure 33). The test revealed no significant differences between transport and handling systems such as railway, road, ship, and oil mill, confirmed by not significant results of ANOVA and GLM.

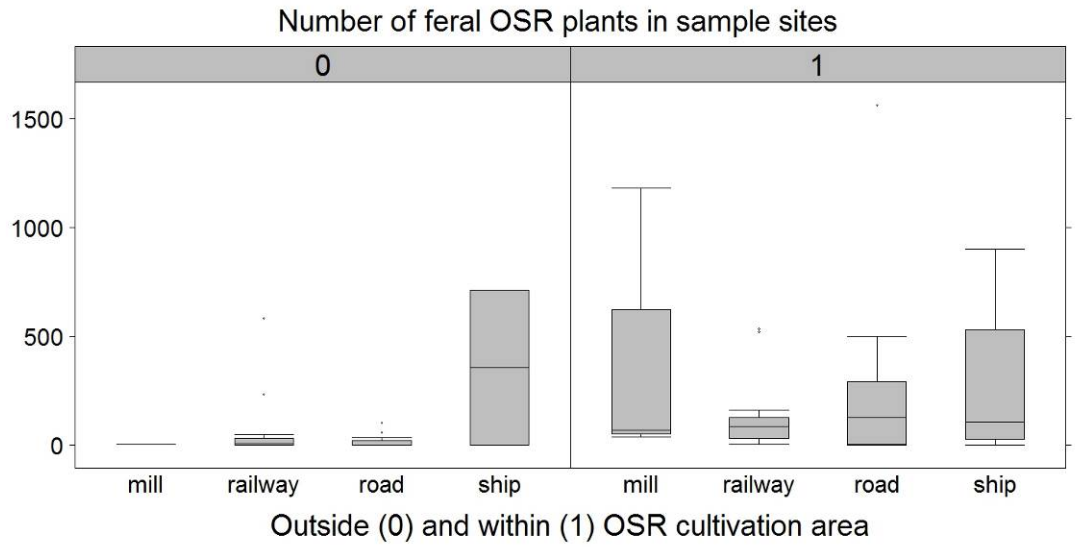


Figure 33: Differences between transport categories concerning number of feral OSR plants.

9.3. Compiled information about the 60 sample sites

Additional information relevant for the 60 sample sites was collected during fieldwork and from homepages of the listed institutions.

Oilseed processing facilities (oil mills and oil processing):

- **Vereinigte Fettwarenindustrie (VFI) GesmbH; Wels (OEM1 in 2014 and aOEM1 in 2015):** VFI Wels (see Figure 34) has its roots in a family company in the sixth generation (http://vfi.co.at). It belongs to the Rauch family. The processed OSR oil is guaranteed to be 100% from Austria (information received from Mag. Rauch). OSR is directly pressed by the farmers and the oil is processed in the VFI. That means no OSR seeds are delivered to the VFI, but only oil. Also in that case, variety specific information of imported ware is unknown. Information should be received by the seed producers. OSR seeds also originate from domestic harvests ("Maschinenring" or private). VFI complies with certificates such as Austria Bio Garantie, IFS, RSPO and KOSHER (homepage: www.vfi-speiseoel.com).

  The VFI plant area itself is sealed to a high extent and very "clean". The feral OSR plants found in 2014 had not descended from transport spillage, but probably had their origin from seeds of a soil mound. This OSR population could not be proven in 2015 anymore.

- **Fandler Oil Mill (OEM2 and aOEM2):** (www.fandler.at, information from René Allmer, Qualitätssicherung: 2014 and another employee)
  Since 1926 the Fandler Oil Mill (see Figure 34) is a family business in the Pöllauer Valley in Styria which is rich in tradition. In October 2012 a new building was constructed where a big variety of cold pressed oils are now presented. The whole plant was newly asphalted in 2011. Sweepers are used for careful cleaning of the areas. Application of herbicides is prohibited. Disinfestation ("Druck-Entwesung") is used. Using this method the eggs of pests are burst. Monitoring of pests is performed regularly. The oil mill processes 77 plant varieties, one organic and one conventional OSR variety. Conventional OSR (Linoleg) comes from Austria to 100%. The mill is in direct contact with the delivering farmers. Contracts complying with quality standards are made. Cultivation of organic OSR is difficult in Austria

and therefore restricted to small amounts. *Brassicogethes aeneus (*Rapsglanzkäfer) represents the biggest problem in this context. Organic OSR from Austria is used according to availability. Testing is implemented by the AGES (Austrian Agency for Health and Food Safety), the rest amounts are imported from Romania for oil processing (Table 19). In 2015, the total amounts of organic OSR could be covered with Austrian seed. No organic OSR imports from Romania were necessary in this year. The trucks deliver OSR seeds in big bags probably coming from the motorways from Hartberg. Most of the deliveries are brought across Fürstenfeld, Kaindorf and Waltersdorf to the Fandler Oil Mill on highways, but not on motorways.

Mr. Allmer provided seed samples from organic OSR from Romania and conventional OSR seed samples. Because of careful management and cleaning, no feral OSR plants were observed in 2014 and only two small plant-rosettes of feral OSR were sampled in 2015 on the whole area of the oil mill.

- **Oil Mill Bunge (OEM3 and aOEM3):**
  (Information given by Mr. Dieter Burger: 2014 and 2015; http://bunge-austria.at/; http://www.bunge-deutschland.de/)
  Bunge is a leading agribusiness and food company. Its locations in Germany and Austria are part of the internationally active Bunge Group (Bunge Limited) which operates globally in the agri- and food business in over 40 countries. 1989 the Oil Mill Bunge in Austria in Bruck an der Leitha (Figure 34) was put into operation. Currently, OSR as well as sunflower seeds are processed: OSR oil and sunflower-oil for foodstuff and extraction grist and crude oil for feeding stuff. The annual capacity of seed processing is 380,000 tons. 10,000 tons fit into the oil tanks. This amount corresponds to loads of approximately 1,000 trucks. 400,000 tons are processes here. Corresponding to this capacity, Bunge is the most important OSR mill in Austria. The oil share is 43 to 44%. The share of grist ("Rapsschrot") is 20%. 80% of the grist is transported *via* truck. Press cake content is around 1,150 t. The OSR oil is delivered to the VFI in Wels (OEM1, see above) for further processing and is bottled there. The processed OSR seed is guaranteed to be GMO-free, but no organic OSR oil is produced by this mill. Hence, the purchase specification is commercial OSR. Storage capacity of seeds is low. OSR seeds are stored for 1.5 years in stock. Until June - before annual harvest is delivered - seeds of the harvest of the preceding year are processed. Specification of colours is done with bleaching. Grist contains higher content of proteins. Prices are judged by the produce exchange in Rotterdam.

  The Austrian farmers harvest the OSR seeds normally in July. They have a contract with the warehouses ("Lagerhäuser"). Beyond the domestic harvest, OSR imports are necessary. They come from Hungary (25%), Slovakia (25%), Czech Republic (<1%), and Slovenia (<1%) (Table 19). In 2015, Austria (mainly from Burgenland and Lower Austria) covered 1/3 of the required OSR seed amount, 2/3 were delivered from abroad. Emission value of dry weight has to be indicated. In the origin region of Austria appropriate locations are chosen for OSR cultivation and delivery. In the opinion of Mr. Burger the prohibition of neonicotinoids could be a driving force to support farmers in their decision not to cultivate OSR in future or to a lesser extent. But no necessary increase of OSR imports was recorded so far.

  No herbicide is applied in the terrain of the plant (pers. comm. Dieter Burger). Only gasification in warehouses against pests - especially *Brassicogethes aeneus (*Rapsglanzkäfer) - is performed. Phostoxin is applied as gas. Careful cleaning of the production plant has to be performed once a year. In 2014, this was done in July, whereas in 2015 it took place in September. For this purpose hexan is used for cleaning the mill.

Mode of transportation: 90% OSR seeds are transported to the Oil Mill Bunge in Bruck an der Leitha *via* trucks. Due to weak implementation of the polluter pays principle and incomplete cost calculations in the transport-sector, road-transport gained 90% of the market shares. Moreover, rail-transport faced difficulties in delivering OSR loads in time. In former times, the railway station in Bruck an der Leitha served as freight railway station. Loading was possible on nine tracks.

Bunge orders the supply of OSR seeds from the international trade and processes the seeds in the oil mill of Bruck an der Leitha to oil which is further processed for OLIO (pers. comm. with staff of OLIO - Spezial Speisefett und Speiseöl GmbH; see Table of supplementary material). Approximately 600 tons of OSR oil per month are bought. Bunge also complies with certificates such as organic ("bio") or RSPO. Import is organised by Bunge Mannheim mainly from Germany, France and the former Eastern European countries such as Hungary and Romania. If insufficient amounts of OSR oil were available, also Australian OSR oil (as oil, not as seeds) would be imported. But also very large amounts of Austrian domestic seeds are processed. Vegetable fat is in some cases imported from Rotterdam, a reloading point where many transports arrive. Also oil facilities in Northern Germany play a role for OLIO. Most transports come to OLIO *via* truck because of cheaper costs and high reliability. In former times also transport *via* railway (Marchtrenk - terminal close to Wels) was performed. There are also waterways connecting to Mannheim and Mainz. Low water situation of retriever-rivers may interfere with the capacity of oil production in oil mills and are therefore unreliable transportation routes. The processed oils of OLIO are mainly used for gastronomy and food industry (http://www.olio.at). According to market prices, it is decided whether oil of sunflowers or OSR is bought. GMO-absence is spot-checked in lots. 00-OSR is generally used. In Slovakia also old varieties complying with an erucic acid content of <2% are in use which are processed in the Slovakian oil mills. OLIO processes bio-fuels out of waste oil. The company OLIO was not sampled in our study because OSR is only handled and processed in oil form.

According to the information of MATTHIAS ZEISSNER from the Bunge Mannheim the imported OSR loads to Austria are exclusively transported *via* trucks. There is no direct water connection to the oil mill in Bruck an der Leitha. In exceptional cases, ship loads can be received from Hungary. In those cases, OSR loads are transported on the River Danube to Vienna and are then reloaded on trucks. The OSR seeds are loosely transported in tipper trucks which are covered against influence of weather. For processing, mainly viable seeds in a kibbled state are delivered. Trucks have to be cleaned according to cleaning procedures depending on the previous loading of the truck. Special cleaning-chemicals are obliged. As the Bunge Mannheim is organising the import for Bunge in Bruck an der Leitha, the Austrian oil mill does not know which truck arrives from which country and if the transport is domestic or coming from abroad. All OSR seed lots are huddled together. OSR seed is regularly transported to Germany on the River Danube with ports of transhipment in use. OSR seed is also transported from Serbia and Romania along the River Danube through Austria. In exceptional cases, OSR grist from Germany is transported *via* the Brenner to Italy. Truck drivers are not instructed in their driving routes from the Oil Mill Bunge in Mannheim. The purchase of OSR bulk mixture is not variety-specific, but complies with quality standards such as oil content and absence of erucic acids, glucosinolates and GM material. Varieties which achieve good harvest results are normally preferred („EU27-Saaten"). Samples of the seed are taken and its quality is checked. GMP-standards exist for feed. In the oil mill of Bruck an der Leitha, food oil as well as half refined products are produced for bio-fuels, sometimes small parts for leather goods. Variety-specific information could be organised from seed distributers such as RWA. Also information about varieties of importing countries could be received from this source. Bunge Mannheim did not give us variety-specific information.

- **Oil Mill Raab, Upper Austria (OEM4 and aOEM4):**
  (Information given by Thomas Raab 2014 and 2015; http://www.oel-muehle.at/)
  This oil mill is directly situated in the Eferdinger Becken (Figure 34). Since 2005, the mill is in operation as a family business and is focussed on the refinement of regional products. In former times organic plant oil was produced for fuels, since 2007 edible oils – currently ten different oils – are obtained. Organic seeds are used to 100%. The whole organic OSR oil – 50 tons – is imported from Romania (Table 19) and Italy in tied up big bags (Figure 35). Certificates are hold with the farmers. Certificates of charges were made in former times. In the course of annual organic controls suppliers as well as the oil mill itself are subject to a strict control. Additionally, the processed oils are routinely inspected for harmful substances and pesticides.

  Feral OSR plants were found in small rosettes which probably trace back to loading activities. Most of them showed pest infestation maybe because the go back to organic varieties.

Figure 34: Oil processing facilities (OEM1-OEM4).

Figure 35: Big bags used for transportation of goods. a) Source: lcpackaging.de; http://lcpackaging.de/sites/lcpackaging.com/files/product_images/product_3_ventilated_big_bags.jpg; b) storage of big bags before processing, Oil Mill Raab (OEM4).

Table 19: Import information of sample sites.

Institution	Importing country	Amount	Importing country	Amount	Importing country	Amount	Importing country	Amount	Together
OEM2: Fandler Oil Mill	Romania	variable	-	-	-	-	-	-	variable
OEM3: Bunge Austria GmbH	Hungary	25%	Slovakia	25%	Czech Republic	<1	Slovenia	<1	50%
OEM4: Oil Mill Raab	Romania	most	Italy	less	-	-	-	-	100%
DH3: Port of Enns	Hungary	little	-	-	-	-	-	-	little

Railway stations

(See Figure 37)

Railway stations outside Austrian OSR cultivation areas

- **Railway station Völs (BHK01 and aBHK01)**
  Völs is situated along the main route of the Austrian rail line Westbahn. Freight trains pass here, only in exceptional cases. They are parked in the station of Völs as it was the case during sampling in 2015. Platforms and railways were recently renewed in the station. *Diplotaxis tenuifolia* which is a relevant hybridisation partner of OSR was frequently occurring along the railway tracks.
- **Railway station Linz Wahring (BHK02 and aBHK02)**
  Transport of goods mainly takes place during the night. There are no OSR transports or loading activities in this switchyard. Some of the tracks of the switchyard – a choice of them – were sprayed with herbicides several weeks ago which effects can be seen in Figure 36. In 2014 several individuals of feral OSR were registered, whereas in 2015 we could not even observe one plant, maybe because the tracks had already been sprayed at sampling time.

Figure 36: Tracks recently sprayed with chemicals in spring 2015 (Linz Wahringer Bahnhof BHK02).

- **Railway station Innsbruck (BHK03 and aBHK03)**
  Mostly, the goods in transit come from Switzerland and France to Innsbruck. There is also a lot of transit traffic along the Brenner road. If a train is defect, it is led to the railway station of Innsbruck and is repaired there. That could probably be a reason why seed spillage occurs and feral plants were found in 2015 although OSR is not loaded or switched in that station.
- **Railway station Hohenems (BHK04 and aBHK04)**
  Between Bludenz und Dornbirn in Vorarlberg, pasturelands are prevailing with interspersed small maize fields. The station of Feldkirch is a switchyard and is in correspondence with Buchs in Switzerland. Feral OSR plants could be observed along the rail line: Dornbirn (-) – Hohenems (+: 1), Götzis (-) – Rankweil (construction site) (-) – Feldkirch (switchyard) (+) – Feldkirch/Ambach: construction site; at sampling time trains did not stop) – Frastanz (tracks in the direction of Bludenz) (+: 3) – Nenzing (-) – Bludenz (+: 11).

Figure 37: Railway stations outside the Austrian OSR cultivation area (BHK01-10).

- **Eastern Railway Station Graz - Ostbahnhof Graz (BHK05 and aBHK05)**
  The Eastern Railway Station in Graz (Ostbahnhof Graz) is a small railway station in Styria. The big station building is put under preservation, but is currently not in use. Railway control is more and more centralised in Austria, so in Styria. In the near future, the railway stations of Graz will be controlled from Villach. Approximately four freight trains pass the station per day, Spielfeld records approximately 50 freight trains.
- **Railway station Frastanz (BHK06 and aBHK06)**
  See railway station Hohenems (BHK04 and aBHK04).
- **Railway station Preding-Wieselsdorf (BHK07 and aBHK07)**
  This station does not belong to the OEBB, but to the GKB (Grazer Köflacher Bahn, http://www.gkb.at/). Staff of the GKB gave the information that the railway tracks have already been sprayed before sampling date.
- **Railway station Bludenz (BHK08 and aBHK08)**
  The trains coming from Lantrach in Switzerland branch out to Wolfurt or Feldkirch.
- **Railway station Dornbirn (BHK09 and aBHK09)**
  Freight trains are passing that station regularly.
- **Railway station Völkermarkt-Kühnsdorf (BHK10 and aBHK10)**
  The railway station is manned, although there are very little train services.

Railway stations within Austrian OSR cultivation areas (see Figure 38)

- **Railway station Wels (BHR01 and aBHR01)**
  The railway station of Wels was sprayed on the sampling day in 2015 with a focus on the main tracks. Several feral OSR plants could be observed along the railway line of the Westbahn, especially in railway stations where the trains slow down or stop (e.g. Linz, Marchtrenk).
- **Railway station Schlüsslberg (BHR02 and aBHR02)**
  The railway station Schlüsslberg is operated by the station in Wels.
- **Railway station Raasdorf (BHR03 and aBHR03)**
  The warehouse close to the railway station in Raasdorf does not trade with OSR (2015). The OSR takeover is situated in Deutsch-Wagram. Feral OSR plants could be observed along the rail line in 2014: Wien Haidestraße (+) – Wien Praterkai (+) – Wien Stadlau (-?) – Erzherzog Karl Straße (+) – Hirschstetten (-) – Wien Hausfeldstraße (-?).
- **Railway station Siebenbrunn-Leopoldsdorf (BHR04 and aBHR04)**
  In 2015, the region besides the railways was mowed. Usually, the warehouse uses a broadband intensive herbicide (registered and approved for the application on railway tracks by the Austrian Agency for Health and Food Safety, AGES) which is applied to the railway tracks. OSR seeds are not transported.
- **Railway station Grieskirchen (BHR05 and aBHR05)**
  Transportation of goods is frequently conducted on line 2. The tracks of the warehouse besides the station were treated with herbicides.
- **Railway station Trautmannsdorf an der Leitha (BHR06 and aBHR06)**
  OSR is handled in Wiener Neustadt. From there OSR is transported to the oil mill or to interim storage. From Wiener Neustadt transportation *via* railways is possible which was not performed in 2014 due to logistic reasons. Hungary is intensely present on the market because of profit reasons. It is assumed that the number of OSR fields in Austria will decrease due to prohibition of neonicotinoids. There was a lot of good traffic observed in 2015. Passenger traffic takes place just once every hour in both directions.

Figure 38: Railway stations within the Austrian OSR cultivation area (BHR01-10).

Feral OSR along the track line was observed in 2014: Grillgasse (+) – Kledering (+) – Lanzendorf/Rannersdorf (+) – Himberg (+) – Gramatneusiedl (+) – and Götzendorf (+) as well as in 2015: Himberg (>) – Grammatneusiedl (>) – Götzendorf (<) – Sarasdorf (+) – Wilfleinsdorf (-) – Bruck an der Leitha (>> Raps).

- **Railway station Wampersdorf (BHR07 and aBHR07)**
- **Railway station Traun (BHR08 and aBHR08)**
- **Railway station Dürnkrut (BHR09 and aBHR09)**
  Herbicide application of the retriever tracks to the warehouse in Dürnkrut was performed at the beginning of May using a broadband herbicide (registered and approved for the application on railway tracks by the Austrian Agency for Health and Food Safety, AGES). Austrian farmers deliver their harvested OSR seeds to Dürnkrut with a truck. In the warehouse it is stored for further transportation. From there it is transported to Bruck an der Leitha to the oil mill as it was also done in December 2014. The warehouse is obliged to spray the tracks.
- **Railway station Hirschstetten-Aspern (BHR10 and aBHR10)**
  No feral OSR plants were found in 2014 on this railway line usually showing frequent OSR spillage. In 2015, one plant could be sampled.

Border railway stations

(See Figure 39)

- **Railway station Nickelsdorf (GBH1 and aGBH1)**
  Nickelsdorf is the adjacent railway station in Austria to the railway station Hegyeshalom at the Hungarian Border. OSR is transported from Hungary to the oil mill of Bunge situated in Bruck an der Leitha. A large number of OSR feral plants grows along the tracks to Nickelsdorf as well as in the railway station of Bruck an der Leitha tracing back to seed spillage. In the station of Parndorf also around 30 OSR individuals could be observed. The same was true for Zurndorf especially on the line in the direction to Bruck an der Leitha. Similar observations could be made in 2015: there were many feral plants in and between the tracks: Parndorf (>) – city of Parndorf (>>) – Zurndorf (> OSR fields). There is a lot of goods transportation along this line, but only minor passenger traffic. Strong winds are regularly observed in Nickelsdorf.
- **Railway station Hohenau (GBH2 and aGBH2)**
  The railway station of Bernhardsthal is situated in Austria, directly at the border to Czech Republic and Slovakia. Especially coal is transported to Czech Republic. Until 1995 (see Chapter 6.1.2.2), when the Austrian borders to the neighbour countries were still checked, the railway station of Hohenau was intensively used for transport of goods.
- **Railway station Baumgarten (GBH3 and aGBH3)**
  This station does not belong to the OEBB, but is part of the Raaberbahn, GYSEV. Until 2008, the Raaberbahn was officially named Raab-Oedenburg-Ebenfurter Eisenbahn (ROeEE) and Hungarian Győr-Sopron-Ebenfurti Vasút (GySEV), respectively. The company is a joint enterprise of the states of Hungary and Austria. Traffic of goods and tanks is conducted. No railway points (“Weiche”) were in the sampling track section. Passenger trains are run once an hour in each direction. There are slopes along the railway tracks which are very rich in grasses (2014). An OSR field was directly adjoined to the railway line in 2014. Field edges would be a possible site for feral OSR to occur. Volunteers from the OSR field which was observed in the last year could be found in 2015. Although feral OSR could not be recognized on the tracks, it was observed along the roads and bridges in that region. These plants probably trace back to OSR seed spillage during transportation by truck.

Figure 39: Border railway stations (GBH1-7). GBH6 Passau was dropped.

- **Railway station Kufstein (GBH4 and aGBH4)**
  The tracks are intensively covered with weeds. In 2014, especially the lines 3, 4 and 5 showed intense grain spillage. Transportation of goods is frequent at that station. Also building material is transported. OSR is freighted to the nearby warehouse from time to time. Goods transportation is performed from Germany to Italy and probably also the other way round.

- **Railway station Marchegg (GBH5 and aGBH5)**
  In former times railways were frequently used for transportation of goods. Nowadays most of the goods are transported *via* trucks. Due to weak implementation of the cost-by-cause principle and incomplete cost calculations (concerning external costs) in the transport-market, the railtransport-sector lost most of its market shares to the roadtransport-sector. This is not in line with political mobility targets and environmental politics, but up to now it is a fact. There is no transportation of grain. Especially materials are ferried. OSR is stored in the warehouse situated closely to the railway station in Marchegg wherefrom trucks transport goods abroad.
- **Railway station Passau (GBH6 and aGBH6a)**
  Because the railway station of Passau belongs to Germany and there is no corresponding railway station at the Austria border, we dropped that site although Passau was the main custom office (73%) of declaration of OSR cargo in 2012 and 2013.
- **Railway station Salzburg (GBH7 and aGBH7)**
  The railway station of Salzburg has already been under construction since 2009. An increasing number of foreign companies is present and fewer tracks are used for transportation than in former times. Hence, several tracks for transportation have been given up in favour of trucks which now transport the bulk of goods (see statements concerning the transport-market above). In 2014, four railway sections were inspected for the occurrence of feral OSR, where transport of goods is carried out. These sections focussed on the main tracks leading into the direction to Vienna and the other way round (line 1 and 2), the turning to Kasern, Hallwang 3, then Liefering (Container-Terminal; Schloss Klessheim, Europapark) and a section leading to Salzburg Taxam. The tracks were sprayed at the beginning of May, several of the frequented tracks were already newly gravelled which means that the number of seeds in the gravel was reduced at that time. Sampling sections in 2015 included the main track leading into the direction to Vienna, the sidings of grain, the tracks into the direction of Gnigl as well as into the direction of Kasern.

Switchyards

(See Figure 40)

- **Switchyard of Kledering (FB1 and aFB1)**
  (http://www.oebb.at)
  The „Zentralverschiebebahnhof Wien-Kledering (Zvbf)" is the biggest switchyard in Austria. Construction work of the switchyard of Kledering started in 1978. Total implementation took place in September 1986. The course of the tracks comprises altogether 120 kilometres. The switchyard of Kledering has a set of arrival sidings which contains 15 tracks. The set of sorting sidings disposes of 48 tracks and the set of departure sidings of 10 tracks. In Eastern direction of the set of sorting sidings, the loop line group with 4 tracks is located. Beyond that, the engine loop line as well as the holding track are resided in Western direction. The main winding hill ("Hauptabrollberg") has a maximal decline of 4.7%. The whole railway track is covered with a calcareous loam layer which prevents running out liquids for infiltration into the soil. After operation of the incoming train („Eingangszug-behandlung") in the set of arrival sidings, the train is disassembled into wagons. Also freight trains are reassembled here. Moreover, trains going abroad are also rearranged. The wagons are moved on top of a small hill and are then released to be separated on different tracks under computer controlled guidance. Within 24 hours, approximately 2,500 to 2,800 wagons are dispatched. The full potential would be 6,000 wagons per day. Controlling and timing is conducted electronically.

Figure 40: Switchyards (FB1 und FB2).

The switchyard of Kledering mainly serves for transportation of goods. Hence, there are no systems for freight handling in that area. Goods terminals are situated in Wien-Inzersdorf and in Wien-Freudenau. In Austria, the transport of OSR seeds on railways is performed in closed wagons. These wagons dispose of funnels to fill in the seeds. At the bottom of the wagons there are tubular outlets. If the unloading hatch (see Figure 41) is porous or not carefully closed, seeds will continuously get lost. Tracks are sprayed once a year in the time span between April and May when the first weeds appear. The second sample site in 2015 focussed on the site where wagons are cleaned inside the cargo bay (see Figure 41). In this cleaning area of wagons seed spillage of OSR will occur, as it was confirmed by findings of OSR plants during sampling in 2015.

Figure 41: Cleaning area of wagons: a) unloading hatch of wagon; b) feral OSR plant tracing back to seed spillage during cleaning.

- **Switchyard of Wels (FB2 and aFB2)**
  From there the Passau Railway ("Passauer Bahn") starts which connects Wels in Upper Austria with Passau situated in Bavaria. From there on it grades into the Passau–Regensburg Railway.

Transportation roads

Transportation roads within Austrian cultivation area (see Figure 43)

- **Road section Katzdorf bei Wallern an der Trattnach (SR01 and aSR01)**
  On this road section there is heavy traffic (2014 and 2015) with a lot of trucks passing that area. In 2015, road sides were mown with a bar mower on the sampling day (see Figure 42).

Figure 42: Vehicle with a bar mower.

- **Road section Aumühle bei Wels; Fraham (SR02 and aSR02)**
  On this heavy traffic road the largest amount of feral OSR plants from all sampled roads has been observed in 2014 with more than estimated 1,400 individuals. Feral OSR prefers to colonise earthy sites especially in outside curves (2015).
- **Road section Waizenkirchen an der Aschach (SR03 and aSR03)**
  There was heavy traffic in that road section with a lot of trucks. Several road sides were recently constructed and mown. The road section contains several curves which facilitate seed spillage. In 2015, road sides had not been mown before sampling. No OSR fields could be observed in the surroundings. No feral OSR was registered indicating that there is no transport activity from OSR fields in that area. In contrast, in the nearby area of Waizenkirchen OSR cultivation was performed during sampling years.
- **Road section Taufkirchen- Leoprechting (SR04 and aSR04)**
  During sampling there was little traffic. The transport road directly leads through small villages. Lots of OSR plants in small rosettes were found between the paving stones, on the traffic islands and planting beds in the small cities which trace back to transport spillage in both sampling years. In 2015, the road sides were already mown and partly in construction. There was also an OSR field in close proximity.
- **Road section Thalmannsbach (SR05 and aSR05)**
  There was little traffic during sampling in 2014 as well as in 2015, but many trucks. Construction work was performed around the petrol station in 2014. In 2015, the sites have already become overgrown.
- **St. Florian am Inn (SR06 and aSR06)**
  Heavy traffic could be observed in 2014 with lots of trucks passing. The road section is leading from the exit of the motorway through the city zone of St. Florian and its industrial areas. The road sides were already mown in 2015. An OSR field beside the road in 2014 changed to a maize field in 2015.
- **Hinding - along the River Danube (SR07 and aSR07)**
  Beside the road there is a big pit of gravel from the Danube which has been intensely covered with a lot of different neophyte species in the sampling years. There is a cycleway in parallel with the highway. Comparatively, there was little traffic. The road sides were already mown. The surroundings were species rich meadows with orchids. There were no fields in close proximity. Hence, the road section belongs to the group of roads within OSR cultivation areas because the community has small parts of OSR cultivation, but not in close proximity to the section.

Figure 43: Sampled sections (2 km) of transportation roads within the Austrian OSR cultivation area (SR01-SR11).

- **Schardenberg - Steinbrunn (SR08 and aSR08)**
  In 2014, the road leading through the two villages was newly tarred. The road was not frequently used by trucks during sampling-time. OSR is cultivated in that area. Heavy traffic could be observed on the road from Passau to Münzkirchen.
- **Pischelsdorf in Styria (SR09 and aSR09)**
  During sampling in 2015, trucks could be observed. The road sides which contained many species typical for meadows were already mown when sampling. A big wood business was situated nearby.
- **Dienersdorf - Kaindorf in Styria (SR10 and aSR10)**
  Several trucks passing could be observed in that road section. Road sides had not been mown or had just partly been cut. They contained many species typical for meadows on sandy, dry soil which could also serve as suitable site for the establishment of feral OSR. Little traffic could be observed during sampling in 2015. Road construction work was partly performed. The road sides were partly mown. There were no OSR fields to be seen around the road section, but maize and grain fields.
- **Bruck an der Leitha (SR11 and aSR11)**
  On this road section OSR is transported from the exit of the motorway to the Oil Mill Bunge. Many feral OSR plants grew within and around the big traffic circle in both sampling years. Moreover, there were earth deposits with *Sisymbrium loeselii*, *Sinapis arvensis*, and feral OSR as well as open areas which serve as potential habitats for feral OSR along the road section.

Transportation roads outside Austrian cultivation area (see Figure 44)

- **Eferding (SK01 and aSK01)**
  A lot of trucks were on their way with medium traffic. In 2015, there were construction works which caused a road block. There was a big difference (22 to 1) of feral OSR plant occurrence, compared in both sampling years.
- **Hilkering near Aschach an der Donau (SK02 and aSK02)**
  There were lots of trucks on their way. In 2015, the road sides were already mown.
- **Pupping and Karling bei Aschach (SK03 and aSK03)**
  A railway line is running parallel to the road. In the majority of cases feral plants were found in solitary occurrence.
- **Engelhartszell - on the River Danube (SK04 and aSK04)**
  Little traffic was observed, although trucks were on their way. A cycleway was in parallel with the highway leading to Passau. The road sides were unmown. However, 2 to 3 feral OSR plants could be observed along the Danube motorway outside the sampled road section.
- **St. Johann im Pongau (SK05 and aSK05)**
  The road section is a highway with heavy traffic. Heavy traffic seems to be usual on the main roads and motorways in that area of Salzburg. The road sides were mown. Sites for the establishment of feral OSR would be present, although no feral OSR was found along the sampled road section. One OSR or turnip plant was observed outside our sampled road section during passing with the car.
- **Bischofshofen (SK06 and aSK06)**
  The selected road section was part of a highway without the possibility to get off the car. During driving no feral OSR plant was observed along the road sides. Hence, a smaller road in parallel to the highway and the River Salzach were investigated for feral OSR plants. One feral OSR plant was registered on the highway in the direction of Villach that means, although infrequent, spillage of OSR seeds is generally possible. Road sides were mown. In 2015, a few feral OSR plants were found under a bridge which perhaps had not been risen from seed spillage but from the earth deposit.

Figure 44: Sampled sections (2 km) of transportation roads outside the Austrian OSR cultivation area (SK01-SK11).

- **Zell am See (SK07 and aSK07)**
  Approximately half of the sampled road section is passing the city of Zell am See. Hence, there were only a few trucks on that road besides intense tourist traffic. In 2015, road sides and traffic circles were mown. Here open sides for the establishment of feral OSR would be present.
- **Maishofen (SK08 and aSK08)**
  There was heavy traffic with lots of cars and a few trucks on the road which is partly tunnelled. Mostly building material is transported. The road sides were mown. Suitable habitats would be present for OSR establishment. There is a dairy farming company in Maishofen. Hence, there are exclusively meadows and pastures in that area. Along all road sections in Salzburg riding stables are very frequent.
- **Schwarzach im Pongau (SK09 and aSK09)**
  There was heavy traffic on this very small road with many trucks on their way. A new tunnel was under construction. There was no path for pedestrians. Also grass verges attending the roads were only partly present which were already mown.
- **Saalfelden am Steinernen Meer (SK010 and aSK010)**
  In 2014, there was heavy traffic. A path is in parallel to the road. Exceptional, there is dairy farming (meadows and pastures) in that region. The road sides were recently mown and disposed meadow character. There would be several potential sites present for feral OSR to grow.
- **Going - in close proximity to the mountain Wilder Kaiser (SK011 and aSK011)**
  In 2014, there was heavy traffic. Dairy farming is prevailing in that region with little agriculture, e.g. maize. The village of Elmau was tunnelled in 2015, just being under construction during sampling. Also the layout of the road was altered. Turnip was growing on the earth deposits of the construction work. The road sides had not been mown when sampled.

Ports along the River Danube (see Figure 45)

- **Port of Albern (DH1 and aDH1)**
  (Information provided by Mr. Buzek; http://www.hafen-wien.com)
  The port of Freudenau is the centre of cargo handling along the River Danube. The handling facilities for bed material load (Schüttgüter) and raw material, the container terminal, a car terminal, halls and storage areas, distribution centres for branded goods, the largest Austrian custom general storage area with custom office and an own police station as well as the direction and the administration of the Viennese port are located here. The port Freudenau also serves as protection and winter port. Around 1,200 tankers are landing on the seven stations of the Ölhafen Lobau per year. Annually, more than 1.2 million tons of mineral oil products are charged. The oil harbour is connected with the central tank depot in the Lobau and the refinery of Schwechat. The freight railway station of the Lobau connects the Ölhafen with the railway net. In the port of Albern (Hafen Albern) construction material, agrarian products, and steel products are charged. There are five large grain silos with a total capacity of altogether 90,000 tons: Getreide Bruck J. & E., RWA, Grandi Molini Handel, Agrarspeicher und Bioprodukte Pinczker. Hence, the port of Albern is one of the leading locations where grain is charged in Eastern Austria.
- **Port of Krems (DH2 and aDH2)**
  (Information kindly provided by Walter Senk von Mierka, Mierka broschure)
  Mierka is a logistic company. "*Mierka Donauhafen Krems can already provide end-to-end trimodal logistics concepts with high quality levels along the 3,500 kilometre long Rhine/Main/Danube waterway in conjunction with the Rhenus Port Logistics business area – from the North to the Black Sea* (broschure "Trimodal logistics" – Mierke Donauhafen Krems"). *The Port Logistics business area has its own warehouse and transshipment facilities and interfaces with rail services at the northern German ports, in Rotterdam,*

Amsterdam and Antwerp and further inland. The company also has operations in Zeebrugge as part of joint venture. Rhenus has local agencies in France, Belgium, Poland, Romania and Bulgaria. Rhenus also has many business locations at inland waterway ports in Germany, Switzerland, France, Luxembourg, Poland, and Romania. Rhenus draws up, manages and operates efficient supply chains at these centres."

Farmers deliver the harvested OSR seeds with trucks to decentralised storage facilities in a radius of around 50 to 70 kilometres. The customer decides the choice of transportation route. The ships in the port of Krems sail to the Netherlands (Spyk, where oil mills are present), Germany (e. g. Straubing in Bavaria), Nois, Mannheim, Rhein-Main). A ship contains loading of approximately 40 to 50 trucks, which are 1,000 to 1,500 tons. The grain silo of the port disposes 25,000 m^3 storage cells. 1m^3 comprises 650 kg OSR. It is not defined which OSR varieties are stored. The storage-cells are cleaned with a brush. They are filled with different kinds of crops. In 2014 at sampling time, 25,000 tons of OSR were stored from the harvest of 2013 and were then shipped. Up to ten ships may anchor in the port of Krems at the same time. They come from whole Europe. No herbicides are applied in the port area. The staff of the port does not make an issue of it, if there are minimal seed contaminations such as a few OSR grains remaining in a storage cell after cleaning. There are overhang pipes for ships as well as for trucks. OSR is also imported from abroad. Sampling 2015: Foam is needed for sealing of loading areas. Embankments are mown and sprayed. A truck is able to load approximately 1,200 tons. One sample is taken every 500 tons (with maize) and is then analysed in the lab. Here it is checked for absence of GM material and herbicides. After loading, the area is cleaned with a brush. Truck-scale is needed for weighing the loaded good. During sampling, trucks from Croatia and Czech Republic were charging their good.

- **Port of Enns (DH3 and aDH3)**
  (Information provided by Mr. Wanger and the manager of the port Enns)
  Considering the extent of area, Enns is the largest port in Austria. Moreover, it is a very modern port. Oilseed rape is transported with trucks and then unloaded. When stored in a silo, OSR has to be cooled because of its sensitivity during storage. After storage the OSR seeds are loaded onto a ship. On our sampling-day, the cooled OSR seeds were transported over a conveyor band onto a Dutch ship. The ship was intended to return to the Netherlands into the direction of Rotterdam, but previously to transport the OSR seeds to the German oil mill in Staubingen in Bavaria which belongs to the AED (German oil mills). Hence, it was organised that the ship did not drive unloaded.
  In the port area of Enns no herbicides are used for removing feral OSR plants. Plant removal, if necessary, is done manually. There is a big 60 m high situated terrace on the grain silo of the port from which the surrounding landscapes can be seen. There are only a few OSR transports which come from Hungary. Export is mainly performed to Germany to the Staubinger Oil Mill. In that modern plant, low spillage of OSR seeds can be expected because new machines are used which are automatic and have closed conveyor-bands. Tracks for transport are sprayed with herbicides.
- **Port of Grein (DH4 and aDH4)**
  This port is especially for passenger transport. In 2014, feral OSR were found along the road descending obviously from truck transport. In 2015, OSR plants were also found on the embankments of the Danube.
- **Port of Obermühl an der Donau (DH5 and aDH5)**
  This port is especially for passenger transport.
- **Port of Wilhering (DH6 and aDH6)**
  This port is especially for passenger transport from one side of the Danube (Ottensheim) to the other side (Wilhering).

Figure 45: Ports along the River Danube (3 with known OSR loading activities: DH1-DH3 and 3 small ports: DH4-DH6).

9.4. Important facts concerning seed spillage during OSR import

9.4.1. Hotspots of seed spillage

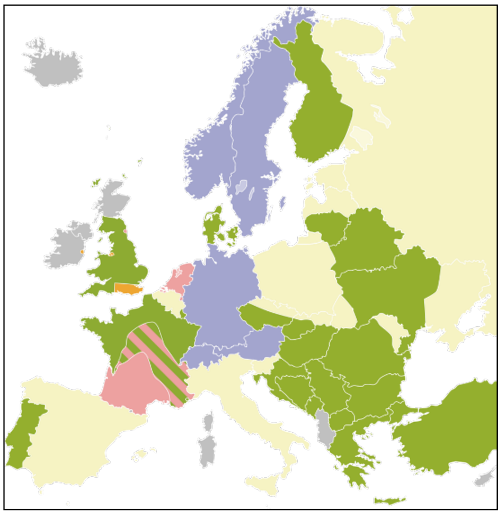
Railway stations at the Austrian border e.g. connecting to Italy

Austria serves as Europe's railway platform which is connected to the railway-network of eight neighbouring countries. More than 100 Mio tons of goods are transported across the Austrian railway tracks. According to container transport, the continuous electrified line from the switchyard Kledering to the Viennese port enables the direct connection to the cargo handling *via* ship and road without extensive switch work (OEBB brochure).

In former times railways were frequently used for transportation of bulk commodities. Due to weak implementation of the cost-by-cause principle and incomplete cost calculations in the transport-sector, road-transport appears to be cheaper than railway-transport. As a consequence, rail cargo-transport lost a main part of its market shares. Hence, most of the goods are transported *via* trucks. The German trains usually pass the Austrian border without stopping, because the trains in these two countries use the same voltage of 15,000 V (15 kV; 16.7 Hz; Figure 46). So does Switzerland and Liechtenstein. However, in Europe different electric current systems do exist. Since the Italian railways normally use 3,000 V (3 KV) for their service, the trains coming from Italy have to slow down at the Austrian border and have to stop there. Hence, seed spillage is more probable at these borders. It was not sensible to sample Darvis or the Brenner which would have been interesting locations for our study, because they are already situated on Italian territory.

Mostly, the goods in transit come from Switzerland and France to Innsbruck. There is also a lot of transit along the Brenner Road. If a train is defect, it stops in the railway station of Innsbruck and is repaired there. Because of slowing down, stopping, handling, and cleaning seed spillage from OSR loadings is probable in that area. Several feral OSR plants could be found on the stabling siding during sampling in 2015 confirming the message of the OEBB staff.

In former times, the OEBB carried out controls of goods wagons at the external frontier to neighbouring countries. During these controls it was also checked whether the unloading hatches of the wagons were properly closed (Figure 41). With the dissolution of the external frontiers in the course of the accession of Austria to the European Union in 1995 January, the 1st, such controls at the Austrian borders started to be discontinued on the internal frontiers which were already valid at that time. With progressive enlargement of the European Union with countries at the Eastern border, the controls were further abandoned. Today, Austria has only a short valid external frontier to Switzerland and Liechtenstein where customs duty and controls are still carried out. Contrastingly, at the internal Austrian EU border wagons with defect or open unloading hatches do not stop at the borders and are not checked - a circumstance which facilitates seed spillage. Hence, it is possible that the transportation goods will spill all over the transportation line.



European systems of electric current of railways.
State: July 2007

orange	750 V
pink	1,500 V
light yellow	3,000 V (3 kV): Italy, Belgium, CIS-states
violet	15,000 V (15 kV): Austria, Germany, Switzerland, Liechtenstein
green	25,000 V
light grey	no electrification

Figure 46: Source: „Europe rail electrification“, Jklamo – own work. Licenced under „unter Gemeinfrei“ over Wikimedia Commons - https://commons.wikimedia.org/wiki/File:Europe_rail_electrification.png#/media/File:Europe_rail_electrification.png

Loading and handling sites of OSR

Especially on sites, where OSR is loaded and handled in a loose form, steady seed spillage can be observed. Ports and OSR processing facilities such as oil mills and processing plants are hotspots for seed spillage. At these sites also imported OSR seed is handled. The study of SCHULZE et al. (2014) in Switzerland confirmed GM OSR spillage at those sites. Different dynamics of GM OSR spread were identified especially in the St. Johann railway station and the Rhine port of Basel. The differences in OSR distribution were correlated with the extent of seed handling and continuous introduction of seeds through spillage.

9.4.2. Weed management

Herbicide management

Virtually, all tracks of our sampled railway stations were sprayed using spraying wagons (orange colour; see Table 20). Mr. Thomas Schuh MSc (“Nachhaltigkeitskoordinator” of the OEBB-Infrastructure Corp.) provided necessary information to us for planning our sampling tour. In 2015, spraying of the sample sites started at the end of May and lasted for approximately eight weeks. The applied agents of the OEBB are registered and approved herbicides in accordance with the register of plant protection (see “Pflanzenschutzmittelregister” of the AGES: http://www.baes.gv.at/pflanzenschutzmittel/pflanzenschutzmittelregister/). The spraying train (“Spritzzug”) is using a detection system to recognize plants along the tracks. Herbicide will only be applied at sites where weeds are growing. With this innovative application technique, OEBB-Infra Corp. managed to reduce the amount of herbicides significantly. Also tracks connecting with warehouses are sprayed with herbicides. For example, in Switzerland at present glyphosate is used as active ingredient for weed management along railway tracks (SCHULZE et al. 2014).

Due to herbicide spraying selection pressure supports survival of feral glyphosate resistant HR GM plants which may lead to an introduction of transgenes into related wild hybridisation partners (LONDO et al. 2010). Consequently, sprayed railway tracks pose an ideal habitat for the potential establishment of GM OSR as it was shown in Switzerland (SCHOENENBERGER & D’ANDREA 2012).

Alternative management

At railway facilities where spraying trains cannot be used, but which would need vegetation management, various other methods are in use. Manuel weeding, mechanical barriers as well as small equipment - e.g. at the switchyard Kledering where the areas between the tracks were mowed with push mowers or mounted spraying equipment - are applied. For a project in Carinthia another alternative method is in experimental stage. Goats are tested for their capability to graze on *Fallopia sp.* which is a big problem on railway tracks.

In contrast to Austria, where most of the goods are transported *via* trucks, Switzerland successfully directs transport from trucks to the railways. On the road sides of the A17 several feral OSR plants could be registered. There are several newly constructed soft shoulders („Bankette“) along that highway. Transportation roads are usually mown with bar mowers (see Figure 43).

In Table 20 also alternatives in weed management are listed. On loading sites in Austria such as ports or OSR processing facilities (oil mills, processing companies) no chemical management is currently in use. In contrast, in Switzerland the ports are sprayed and hence, long-term persistence of glyphosate resistant GM OSR seems to be very likely (Schulze et al. 2014).

Table 20: Applied weed-management on sample sites.

Institution	Management	Used herbicide
OEM1: VFI Wels	very clean; no processing of seeds, just oil	no
OEM2: Fandler Oil Mill	sweepers, disinfestation	no
OEM3: Bunge Austria GmbH	gasification of warehouses (Phostoxin)	-
OEM4: Oil Mill Raab	no management of weeds: organic farming	no
Railway stations	application with "spraying trains" from 27.04. to 28.05.2015	herbicides*
BHK01 - BHK10	spraying of herbicides	herbicides*
BHK02: Linz, Wahring	spraying of organic herbicides with big amounts of fertilizer	organic
BHR01 - BHR10	spraying of herbicides	herbicides*
Railway station Villach	goat project: goats feed on *Fallopia japonica*	no
FB1: Switchyard of Kledering	spraying one a year between April and May	yes
FB1: Switchyard of Kledering	hand mowing of areas between tracks	no
FB1: Switchyard of Kledering	cleaning wagons of seeds with a brush	?
BHR04: Warehouse of Siebenbrunn-Leop.	spraying of an effective herbicide on the tracks	clinic glyphosat
BHR05: Warehouse of Griesskirchen	spraying of the tracks	Roundup-Ready
BHR09: Warehouse of Dürnkrut	spraying of tracks	similar to Roundup Ready
DH2: Port of Krems	port area: no herbicides are applied	no
DH2: Port of Krems	storage cells are cleaned with a brush	no
DH3: Port of Enns	port area: manual removement of plants	no
DH3: Port of Enns	loader tracks are sprayed	yes
DH3: Port of Enns	manual removal of feral OSR plants	no
Transportation roads SR01-SR011 and	vehicle with a bar mower	no

* regulatory permitted herbicides for application to track systems (see Pflanzenschutzmittelregister of the AGES: http://www.baes.gv.at/pflanzenschutzmittel/pflanzenschutzmittelregister/)

9.4.3. Cleaning of transporters

As shown in Figure 47 cleaning of trucks is performed roughly with a brush by the driver himself. A similar situation is true for train wagons.

Figure 47: Cleaning of trucks.

9.4.4. Contamination of wheat seeds as source for feral GM OSR

In Switzerland, the GM OSR events GT73, MS8×RF3, MS8 and RF3 have recently been found in the Rhine port of Basel, although cultivation as well as import of GM OSR (*Brassica napus* L.) are banned in Switzerland. In the paper of SCHULZE et al. (2015) the sources of GM oilseed rape seeds were now identified. Dating back to wheat which are the main agricultural goods being imported at the Rhine port of Basel – from 2010 to 2013, 19% of imports originating from Canada – the feral GM plants could have their origin in imports of Canadian wheat contaminated with OSR. They may contain a low level of impurities of GM OSR (LLP in wheat imported from Canada is estimated to be 0.005% at average). GM OSR is grown in Canada up to 90% of total Canadian OSR cultivation. Moreover, GT73, MS8×RF3, MS8 and RF3 OSR were further identified in processing facilities of two grain mills.

In 2012 Canada produced around 27 Mio tons of wheat and exported 16.3 Mio tons of those to 70 countries. Following USA, France, and Australia, Canada holds an important position on the world market (HUYGEN et al. 2003). Concerning wheat, Austria disposes of a high degree of self-supply (PASCHER 2013). In 2011/2012 import of wheat inclusively processing products in grain equivalent ("Getreideäquivalent") amounted to 710.815 tons and export to 798.522 tons. Canada exported no wheat to Austria from 2010-2012 (FAOSTAT: http://faostat.fao.org/; 15th October 2015).

Concluding, currently no Canadian wheat is imported to Austria. Hence, Canadian wheat is no source for GM OSR contamination at present. Currently, in the port of Enns in Upper Austria wheat is exclusively imported from the Danube region. Only Serbia, Slovakia and Hungary are exporting wheat *via* the port of Enns to Austria (pers. information of Mr. Wanger). In the port of Krems there are null to very little amounts of wheat imported to our country (pers. information of Mr. Senk from Mierka). Austria is a wheat exporter. High quality wheat is mostly exported to Italy. In the port of Albern wheat is regularly handled (pers. communication of Alois Harras-Leben from the RWA). Canadian wheat is mostly imported to those European countries which have access to the sea such as Italy and the Netherlands. The onward movement of Canadian wheat into landlocked countries would result in high transportation costs. For whatever reason, Switzerland is an exception (SCHULZE et al. 2015). But, if Canadian wheat was imported to Austria in the future similar to the current Swiss situation, unintended introduction of GM OSR seeds coming from this route would need to be considered as well.

9.5. Simple Sequence Repeats (SSR) analyses

Locus by locus descriptive statistics for commercial varieties *versus* feral populations are given in Table 21. Although all indices except the normalized Shannon Diversity Index, I_{nor}, suggest that feral populations have higher genetic diversity than the commercial varieties, none of these differences is statistically significant (p-values for the reduced data set given in parentheses): effective number of alleles, n_e: p=0.636 (0.730); observed heterozygosity, H_O: p=0.986 (1); expected heterozygosity, H_E: p=0.636 (0.733); normalized Shannon Index, I_{nor}: p=0.591 (0.559); allelic richness, AR: p=0.142 (0.166).

Table 21: Locus by locus descriptive statistics for commercial varieties and feral populations.

Locus Name	Allele Length	Sample Size	n_a	n_e	H_O	H_E	I_{nor}	AR
Commercial varieties								
Na12_A08	151–183	217	9	1.846	0.194	0.459	0.183	7.406 (8.916)
Na12_C06	206–212	217	4	2.084	0.180	0.521	0.169	3.788 (3.999)
Na12_E01a	222–236	217	7	3.132	0.184	0.682	0.225	6.320 (6.998)
Na12_E01b	246–258	117	5	1.862	0.034	0.465	0.192	5.000
Na12_C08	278–346	217	13	4.172	0.341	0.762	0.315	11.537 (12.943)
NA12_Eo6a	103–125	215	7	1.365	0.223	0.268	0.111	5.862 (6.962)
Na12_C12	240–256	212	7	1.633	0.127	0.389	0.162	6.544 (6.995)
Na10_C01a	213–215	217	2	1.042	0.041	0.041	0.019	1.999 (2.000)
Na10_C01b	247–259	217	4	1.113	0.060	0.102	0.046	3.537 (3.972)
Na12_D11	342–356	211	7	1.311	0.104	0.238	0.103	6.157 (7.000)
Mean		*205.7 (215.6)*	*6.5 (6.7)*	*1.956 (1.966)*	*0.149 (0.162)*	*0.393 (0.385)*	*0.152 (0.148)*	*5.815 (6.643)*
S.D.		*31.2 (2.4)*	*3.1 (3.2)*	*0.986 (1.045)*	*0.095 (0.092)*	*0.234 (0.247)*	*0.087 (0.095)*	*2.597 (3.182)*
Feral populations								
Na12_A08	147–187	1853	16	2.204	0.238	0.546	0.163	9.184 (10.404)
Na12_C06	192–234	1853	18	2.987	0.185	0.665	0.162	7.075 (8.856)
Na12_E01a	220–242	1854	12	3.279	0.217	0.695	0.176	6.424 (7.208)
Na12_E01b	244–274	888	11	2.693	0.064	0.629	0.193	8.967
Na12_C08	264–348	1845	29	6.004	0.409	0.834	0.279	14.525 (16.116)
NA12_Eo6a	103–129	1833	11	1.657	0.216	0.397	0.108	6.475 (7.671)
Na12_C12	228–280	1850	16	1.749	0.100	0.428	0.129	9.255 (10.695)
Na10_C01a	213–217	1855	3	1.044	0.018	0.042	0.014	2.058 (2.114)
Na10_C01b	231–277	1852	9	1.063	0.016	0.060	0.023	3.784 (4.648)
Na12_D11	338–354	1792	9	1.198	0.060	0.165	0.059	6.718 (7.763)
Mean		*1747.5 (1843.0)*	*13.4 (13.7)*	*2.388 (2.354)*	*0.152 (0.162)*	*0.446 (0.426)*	*0.131 (0.124)*	*7.447 (8.386)*
S.D.		*302.6 (20.3)*	*7.0 (7.3)*	*1.501 (1.588)*	*0.124 (0.127)*	*0.278 (0.287)*	*0.082 (0.086)*	*3.396 (3.952)*

n_a = observed number of alleles; n_e = effective number of alleles (Brown and Weir 1983); H_O = observed heterozygosity; H_E = unbiased expected heterozygosity (NEI 1978); I_{nor} = Shannon's Diversity Index normalized by sample size, i.e., I_{nor}=I/ln(sample size); AR = Allelic Richness calculated using the rarefaction method of EL MOUSADIK & PETIT (1996).
Values are given for the entire data set (including 10 loci) and, in parentheses, for the reduced data set (9 loci, excluding Na12_E01b due to a high amount of missing data).

Population level descriptive statistics are given in Table 22. Feral populations had higher levels of genetic diversity which were statistically highly significant (*p*<0.001) except for observed heterozygosity (*p*=0.108 and *p*=0.124 for the complete and the reduced data set, respectively). Population groups (railway stations, switchyards, border railway stations, ports, road sections, oil processing facilities – oil mills and OSR processing company) showed different levels of genetic diversity, but the rank of each group differed between different diversity measures (and occasionally also between the complete and the reduced data sets). For instance, switchyards had the highest percentage of polymorphic loci, but only medium levels of observed heterozygosity. Differences in genetic diversity were statistically not significant (p-values for the reduced data set given in parentheses): effective number of alleles, n_e: *p*=0.355 (0.246); percentage of polymorphic loci, Per_{poly}: *p*=0.468 (0.471); expected heterozygosity, H_E: *p*=0.375 (0.222); normalized Shannon Index, I_{nor}: *p*=0.302 (0.118). The only exception was observed heterozygosity, H_O, which was significant for the complete but not the reduced data set: *p*=0.038 (0.079). However, although in pairwise Mann-Whitney tests several cases of significant differences were found (e.g. train stations *versus* harbours and *versus* roadsides), these became non-significant after sequential Bonferroni correction.

Table 22: Descriptive genetic statistics for feral OSR populations and for commercial OSR varieties.

	Sample Size	Avg. No. Data / Locus	N_{GT}	n_a	n_e	Per_{poly}	H_O	H_E	I_{nor}
Feral OSR Populations									
(BH)									
BHK1	3	3.00 (3.00)	3 (3)	1.400 (1.444)	1.340 (1.378)	40.00 (44.44)	0.167 (0.185)	0.220 (0.244)	0.237 (0.263)
BHK2	36	34.30 (35.78)	34 (34)	4.200 (4.444)	1.848 (1.919)	100.00 (100.00)	0.145 (0.161)	0.367 (0.388)	0.203 (0.214)
BHK3	27	25.60 (26.67)	26 (26)	3.600 (3.667)	2.067 (2.071)	100.00 (100.00)	0.223 (0.247)	0.488 (0.484)	0.262 (0.258)
BHK4	1	1.00 (1.00)	1 (1)	1.100 (1.111)	1.100 (1.111)	10.00 (11.11)	0.100 (0.111)	0.100 (0.111)	0.000 (0.000)
BHK6	1	1.00 (1.00)	1 (1)	1.200 (1.111)	1.200 (1.111)	20.00 (11.11)	0.200 (0.111)	0.200 (0.111)	0.000 (0.000)
BHK8	10	9.40 (9.89)	10 (10)	2.700 (2.889)	1.817 (1.908)	70.00 (77.78)	0.172 (0.191)	0.358 (0.398)	0.268 (0.297)
BHR1	68	63.10 (67.56)	67 (67)	6.300 (6.556)	2.515 (2.582)	100.00 (100.00)	0.177 (0.187)	0.466 (0.464)	0.241 (0.235)
BHR10	1	0.90 (1.00)	1 (1)	1.100 (1.222)	1.100 (1.222)	20.00 (22.22)	0.200 (0.222)	0.200 (0.222)	0.000 (0.000)
BHR2	12	11.00 (11.56)	12 (12)	3.400 (3.444)	2.224 (2.175)	90.00 (88.89)	0.148 (0.146)	0.491 (0.470)	0.368 (0.345)
BHR3	23	21.50 (23.00)	19 (19)	3.200 (3.333)	2.108 (2.133)	90.00 (88.89)	0.222 (0.246)	0.427 (0.419)	0.247 (0.239)
BHR4	56	52.10 (55.89)	40 (39)	4.700 (4.778)	1.958 (1.877)	100.00 (100.00)	0.110 (0.103)	0.383 (0.353)	0.201 (0.179)
BHR5	48	44.70 (47.00)	47 (45)	5.400 (5.333)	2.214 (2.204)	100.00 (100.00)	0.148 (0.159)	0.447 (0.432)	0.240 (0.228)
BHR6	28	26.60 (27.78)	28 (28)	4.800 (4.556)	2.540 (2.401)	90.00 (88.89)	0.209 (0.218)	0.459 (0.425)	0.291 (0.259)
BHR7	11	10.40 (11.00)	11 (11)	2.700 (2.667)	1.951 (1.915)	70.00 (66.67)	0.182 (0.202)	0.381 (0.354)	0.290 (0.257)
BHR8	26	23.70 (26.00)	26 (26)	3.500 (3.778)	1.976 (2.084)	80.00 (88.89)	0.150 (0.167)	0.354 (0.394)	0.211 (0.234)

BHR9	53	50.20 (52.89)	51 (50)	3.600 (3.667)	1.998 (1.998)	80.00 (77.78)	0.191 (0.212)	0.384 (0.370)	0.179 (0.172)
Total	*404*	*378.50 (401.00)*	*364 (350)*						
Mean	*25.3*	*23.66 (25.06)*	*23.56 (23.31)*	*3.306 (3.375)*	*1.872 (1.881)*	*72.50 (72.92)*	*0.171 (0.179)*	*0.358 (0.352)*	*0.249 (0.245)*
(FB)									
FB1	155	147.50 (153.22)	131 (125)	6.500 (6.333)	2.401 (2.419)	100.00 (100.00)	0.183 (0.188)	0.429 (0.415)	0.181 (0.175)
FB2	68	64.10 (67.11)	65 (64)	5.400 (5.444)	2.395 (2.387)	100.00 (100.00)	0.148 (0.153)	0.456 (0.440)	0.220 (0.211)
Total	*223*	*211.60 (220.33)*	*193 (183)*						
Mean	*111.5*	*105.80 (110.17)*	*98.00 (94.50)*	*5.950 (5.889)*	*2.398 (2.403)*	*100.00 (100.00)*	*0.166 (0.171)*	*0.443 (0.427)*	*0.201 (0.193)*
(GBH)									
GBH1	71	67.30 (70.56)	71 (71)	5.200 (5.222)	2.539 (2.547)	100.00 (100.00)	0.170 (0.178)	0.478 (0.464)	0.228 (0.221)
GBH2	95	87.70 (94.44)	90 (89)	5.800 (5.889)	2.412 (2.501)	100.00 (100.00)	0.200 (0.214)	0.430 (0.435)	0.207 (0.203)
GBH3	3	2.70 (2.78)	2 (2)	1.500 (1.556)	1.457 (1.508)	40.00 (44.44)	0.400 (0.444)	0.293 (0.326)	0.242 (0.273)
GBH4	19	17.20 (18.78)	11 (11)	2.800 (3.000)	1.525 (1.583)	90.00 (100.00)	0.112 (0.125)	0.285 (0.317)	0.176 (0.196)
GBH5	68	64.40 (67.89)	65 (63)	4.400 (4.333)	2.342 (2.241)	90.00 (88.89)	0.133 (0.144)	0.445 (0.417)	0.209 (0.190)
GBH7	3	2.70 (2.89)	3 (3)	1.700 (1.778)	1.454 (1.505)	60.00 (66.67)	0.150 (0.167)	0.297 (0.330)	0.412 (0.412)
Total	*259*	*242.00 (257.33)*	*238 (233)*						
Mean	*43.2*	*40.33 (42.89)*	*40.33 (39.83)*	*3.567 (3.630)*	*1.955 (1.981)*	*80.00 (83.33)*	*0.194 (0.212)*	*0.371 (0.381)*	*0.246 (0.249)*
(DH)									
DH1	87	81.50 (85.89)	85 (81)	6.300 (6.444)	2.386 (2.361)	100.00 (100.00)	0.150 (0.159)	0.452 (0.432)	0.215 (0.206)
DH2	114	107.80 (113.11)	111 (106)	6.300 (6.333)	2.313 (2.252)	100.00 (100.00)	0.135 (0.144)	0.454 (0.432)	0.198 (0.186)
DH3	60	55.80 (59.00)	58 (56)	5.200 (5.333)	2.333 (2.240)	90.00 (88.89)	0.127 (0.137)	0.448 (0.420)	0.224 (0.207)
DH4	39	37.60 (38.89)	32 (28)	3.400 (3.111)	1.865 (1.784)	70.00 (66.67)	0.081 (0.085)	0.324 (0.290)	0.175 (0.151)
Total	*300*	*282.70 (296.89)*	*260 (239)*						
Mean	*75.0*	*70.68 (74.22)*	*71.50 (67.75)*	*5.300 (5.306)*	*2.225 (2.159)*	*90.00 (88.89)*	*0.123 (0.131)*	*0.419 (0.394)*	*0.203 (0.187)*
(S)									
SKO1	23	21.60 (22.89)	23 (23)	4.300 (4.333)	2.086 (1.965)	100.00 (100.00)	0.164 (0.171)	0.446 (0.416)	0.289 (0.260)
SKO2	52	49.50 (51.89)	46 (39)	3.900 (3.667)	1.757 (1.632)	80.00 (77.78)	0.110 (0.122)	0.298 (0.257)	0.157 (0.130)

SKO3	18	17.10 (18.00)	17 (14)	3.100 (3.111)	1.518 (1.467)	90.00 (88.89)	0.122 (0.136)	0.303 (0.279)	0.199 (0.178)
SR11	84	78.60 (82.67)	80 (78)	5.700 (5.556)	2.309 (2.305)	100.00 (100.00)	0.138 (0.150)	0.438 (0.422)	0.207 (0.195)
SRO1	55	52.30 (54.89)	45 (40)	3.800 (3.778)	1.803 (1.729)	90.00 (88.89)	0.078 (0.079)	0.342 (0.313)	0.169 (0.151)
SRO2	67	63.50 (66.89)	59 (52)	4.900 (4.556)	2.022 (1.872)	100.00 (100.00)	0.097 (0.105)	0.389 (0.352)	0.194 (0.167)
SRO4	59	56.00 (58.56)	54 (53)	4.800 (4.667)	2.194 (2.106)	100.00 (100.00)	0.111 (0.124)	0.437 (0.411)	0.221 (0.203)
SRO5	59	55.30 (58.33)	55 (51)	5.600 (5.556)	2.404 (2.245)	100.00 (100.00)	0.137 (0.152)	0.441 (0.406)	0.229 (0.204)
SRO6	27	25.70 (26.78)	26 (26)	3.600 (3.556)	2.056 (2.000)	90.00 (88.89)	0.190 (0.211)	0.419 (0.395)	0.239 (0.223)
SRO8	17	15.50 (16.89)	16 (16)	2.700 (2.778)	1.940 (1.955)	70.00 (66.67)	0.210 (0.233)	0.369 (0.351)	0.257 (0.222)
SRO9	3	3.00 (3.00)	3 (3)	1.600 (1.667)	1.477 (1.530)	50.00 (55.56)	0.033 (0.037)	0.287 (0.319)	0.324 (0.360)
Total	*464*	*438.10 (460.78)*	*349 (314)*						
Mean	*42.2*	*39.83 (41.89)*	*38.55 (35.91)*	*4.000 (3.929)*	*1.961 (1.891)*	*88.18 (87.88)*	*0.126 (0.138)*	*0.379 (0.356)*	*0.226 (0.208)*
(OEM)									
OEM1	21	19.30 (20.89)	21 (21)	3.200 (3.333)	1.805 (1.842)	100.00 (100.00)	0.179 (0.198)	0.397 (0.401)	0.242 (0.234)
OEM2	3	2.60 (2.89)	3 (3)	2.200 (2.444)	1.921 (2.135)	80.00 (88.89)	0.233 (0.259)	0.487 (0.541)	0.699 (0.699)
OEM3	151	141.60 (150.11)	141 (136)	7.200 (7.222)	2.310 (2.306)	100.00 (100.00)	0.169 (0.177)	0.435 (0.420)	0.188 (0.179)
OEM4	33	31.10 (32.78)	32 (32)	4.300 (4.333)	2.182 (2.122)	100.00 (100.00)	0.193 (0.186)	0.464 (0.443)	0.255 (0.238)
Total	*208*	*194.60 (206.67)*	*194 (188)*						
Mean	*52.0*	*48.65 (51.67)*	*49.25 (48.00)*	*4.225 (4.333)*	*2.055 (2.101)*	*95.00 (97.22)*	*0.193 (0.205)*	*0.446 (0.451)*	*0.346 (0.337)*
Grand Total	*1858*	*1747.50 (1843.00)*	*1414 (1294)*						
Grand Mean	*43.2*	*40.64 (42.86)*	*40.05 (38.58)*	*3.914 (3.938)*	*1.981 (1.968)*	*82.56 (83.20)*	*0.160 (0.171)*	*0.383 (0.374)*	*0.245 (0.236)*
Commercial Varieties									
Adriana	5	4.50 (10.00)	2 (2)	1.000 (1.111)	0.992 (1.103)	10.00 (11.11)	0.000 (0.000)	0.053 (0.059)	0.046 (0.046)
Alabaster	5	5.00 (10.00)	2 (2)	1.500 (1.556)	1.430 (1.478)	40.00 (44.44)	0.380 (0.422)	0.229 (0.254)	0.187 (0.207)
Albatros	5	4.90 (10.00)	3 (3)	1.400 (1.333)	1.325 (1.294)	40.00 (33.33)	0.240 (0.267)	0.198 (0.173)	0.163 (0.136)
Amethyst	5	4.50 (9.78)	4 (4)	1.600 (1.667)	1.358 (1.398)	50.00 (55.56)	0.130 (0.144)	0.222 (0.246)	0.215 (0.215)
Artoga	5	5.00 (10.00)	3 (3)	1.500 (1.444)	1.370 (1.308)	40.00 (33.33)	0.160 (0.178)	0.207 (0.170)	0.173 (0.146)
Californium	5	4.50 (10.00)	2 (2)	1.000 (1.111)	0.922 (1.024)	10.00 (11.11)	0.020 (0.022)	0.020 (0.022)	0.022 (0.022)

Caracas	4	4.00 (8.00)	2 (2)	1.100 (1.111)	1.060 (1.067)	10.00 (11.11)	0.000 (0.000)	0.043 (0.048)	0.041 (0.045)
Carouser	5	5.00 (10.00)	1 (1)	1.100 (1.111)	1.022 (1.024)	10.00 (11.11)	0.020 (0.022)	0.020 (0.022)	0.020 (0.022)
Casoar	5	5.00 (10.00)	2 (2)	1.100 (1.111)	1.092 (1.103)	10.00 (11.11)	0.000 (0.000)	0.053 (0.059)	0.042 (0.046)
Castille	5	5.00 (10.00)	2 (2)	1.100 (1.111)	1.047 (1.052)	10.00 (11.11)	0.000 (0.000)	0.036 (0.040)	0.031 (0.035)
Digger	5	4.50 (10.00)	3 (3)	1.100 (1.222)	0.969 (1.077)	20.00 (22.22)	0.020 (0.022)	0.056 (0.062)	0.057 (0.057)
DK Excellium	5	4.80 (9.56)	3 (3)	1.500 (1.556)	1.463 (1.515)	40.00 (44.44)	0.360 (0.400)	0.240 (0.267)	0.213 (0.237)
DK Exfiels	5	5.00 (10.00)	2 (2)	1.300 (1.333)	1.292 (1.325)	30.00 (33.33)	0.280 (0.311)	0.164 (0.183)	0.128 (0.142)
DK Expertise	5	5.00 (10.00)	4 (4)	2.000 (2.000)	1.639 (1.658)	70.00 (66.67)	0.340 (0.378)	0.351 (0.351)	0.309 (0.309)
DK Expower	5	4.90 (10.00)	4 (4)	2.000 (2.000)	1.523 (1.550)	80.00 (77.78)	0.365 (0.378)	0.332 (0.341)	0.296 (0.298)
DK Exstorm	5	4.90 (10.00)	3 (3)	2.000 (2.000)	1.636 (1.640)	60.00 (55.56)	0.300 (0.333)	0.318 (0.306)	0.298 (0.286)
DK Sedona	5	4.60 (10.00)	3 (3)	1.700 (1.778)	1.562 (1.625)	50.00 (55.56)	0.460 (0.511)	0.289 (0.321)	0.269 (0.269)
DK Sequoia	5	4.50 (10.00)	1 (1)	1.200 (1.333)	1.200 (1.333)	30.00 (33.33)	0.300 (0.333)	0.167 (0.185)	0.144 (0.144)
Fander (OEM2) Bioraps	5	4.60 (9.78)	5 (5)	2.100 (2.222)	1.750 (1.834)	50.00 (55.56)	0.160 (0.178)	0.304 (0.338)	0.295 (0.328)
Freddy	5	5.00 (10.00)	3 (3)	1.200 (1.222)	1.127 (1.141)	10.00 (11.11)	0.000 (0.000)	0.062 (0.069)	0.059 (0.066)
Gloria	5	4.60 (10.00)	3 (3)	2.300 (2.333)	1.701 (1.667)	80.00 (77.78)	0.380 (0.311)	0.418 (0.353)	0.339 (0.339)
Graf	5	4.90 (10.00)	4 (4)	2.400 (2.333)	2.033 (1.963)	70.00 (66.67)	0.370 (0.356)	0.451 (0.422)	0.427 (0.392)
Harry	5	4.50 (10.00)	1 (1)	0.900 (1.000)	0.900 (1.000)	0.00 (0.00)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Henry	3	3.00 (6.00)	1 (1)	1.000 (1.000)	1.000 (1.000)	0.00 (0.00)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Jimmy	5	4.50 (10.00)	2 (2)	1.000 (1.111)	0.992 (1.103)	10.00 (11.11)	0.000 (0.000)	0.053 (0.059)	0.046 (0.046)
Janus	5	4.50 (10.00)	2 (2)	1.000 (1.111)	0.947 (1.052)	10.00 (11.11)	0.000 (0.000)	0.036 (0.040)	0.035 (0.035)
Jolly	3	3.00 (6.00)	2 (2)	1.100 (1.111)	1.080 (1.089)	10.00 (11.11)	0.000 (0.000)	0.053 (0.059)	0.058 (0.064)
Kutiba	5	4.90 (10.00)	3 (3)	1.200 (1.222)	1.094 (1.105)	20.00 (22.22)	0.000 (0.000)	0.071 (0.079)	0.062 (0.069)
Ladoga	5	4.50 (10.00)	4 (4)	1.200 (1.333)	1.016 (1.129)	30.00 (33.33)	0.020 (0.022)	0.091 (0.101)	0.092 (0.092)
Lenny	5	5.00 (10.00)	3 (3)	1.200 (1.222)	1.094 (1.105)	20.00 (22.22)	0.000 (0.000)	0.071 (0.079)	0.062 (0.069)
Mickey	5	4.50 (10.00)	1 (1)	0.900 (1.000)	0.900 (1.000)	0.00 (0.00)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
NK Petrol	5	5.00 (10.00)	4 (4)	2.000 (2.111)	1.863 (1.959)	80.00 (88.89)	0.640 (0.711)	0.449 (0.499)	0.371 (0.412)
Orlando	5	4.80 (10.00)	5 (5)	1.700 (1.778)	1.439 (1.488)	60.00 (66.67)	0.240 (0.267)	0.258 (0.286)	0.221 (0.245)
Pedro	5	4.80 (10.00)	4 (4)	1.400 (1.444)	1.297 (1.330)	30.00 (33.33)	0.020 (0.022)	0.153 (0.170)	0.135 (0.150)

Peter 29	5	5.00 (10.00)	1 (1)	1.400 (1.444)	1.400 (1.444)	40.00 (44.44)	0.400 (0.444)	0.222 (0.247)	0.172 (0.191)
Remy	5	4.70 (9.56)	2 (2)	1.100 (1.111)	1.022 (1.024)	10.00 (11.11)	0.020 (0.022)	0.020 (0.022)	0.020 (0.022)
Ricky	5	4.50 (10.00)	2 (2)	1.000 (1.111)	0.947 (1.052)	10.00 (11.11)	0.000 (0.000)	0.036 (0.040)	0.035 (0.035)
Sammy	5	4.00 (8.89)	3 (3)	1.000 (1.111)	0.985 (1.094)	20.00 (22.22)	0.000 (0.000)	0.107 (0.119)	0.105 (0.105)
Sherlock	5	4.50 (10.00)	3 (3)	1.100 (1.222)	1.027 (1.141)	10.00 (11.11)	0.000 (0.000)	0.062 (0.069)	0.066 (0.066)
Sherpa	5	4.50 (10.00)	3 (3)	1.400 (1.556)	1.295 (1.439)	40.00 (44.44)	0.260 (0.289)	0.211 (0.235)	0.199 (0.199)
Sidney	5	4.30 (9.56)	1 (1)	0.900 (1.000)	0.900 (1.000)	0.00 (0.00)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
ES Solist	5	5.00 (10.00)	2 (2)	1.400 (1.444)	1.338 (1.376)	30.00 (33.33)	0.300 (0.333)	0.176 (0.195)	0.145 (0.161)
Tenno	3	2.70 (6.00)	3 (3)	1.500 (1.667)	1.380 (1.533)	50.00 (55.56)	0.400 (0.444)	0.293 (0.326)	0.362 (0.362)
Tommy	5	5.00 (10.00)	3 (3)	1.200 (1.222)	1.069 (1.077)	20.00 (22.22)	0.020 (0.022)	0.056 (0.062)	0.051 (0.057)
Top	4	3.80 (8.00)	4 (4)	1.500 (1.556)	1.315 (1.350)	30.00 (33.33)	0.075 (0.083)	0.175 (0.194)	0.178 (0.197)
Total	*217*	*205.70 (431.11)*	*116 (107)*						
Mean	*4.8*	*4.57 (9.58)*	*2.67 (2.67)*	*1.362 (1.420)*	*1.240 (1.290)*	*30.00 (31.61)*	*0.148 (0.161)*	*0.152 (0.159)*	*0.137 (0.141)*

Avg. No. Data / Locus = average number of data per locus; N_{GT} = number of genotypes; n_a = observed number of alleles; n_e = effective number of alleles (BROWN & WEIR 1983); Per_{poly} = percentage of polymorphic loci; H_O = observed heterozygosity; H_E = unbiased expected heterozygosity (NEI 1978); I_{nor} = Shannon's Diversity Index normalized by sample size, i.e., $I_{nor}=I/\ln$ (sample size).
Values are given for the entire data set (including 10 loci) and, in parentheses, for the reduced data set (9 loci, excluding Na12_E01b due to a high amount of missing data).

The two-level AMOVA (Analysis of Molecular Variance; treating the six population groups as populations) assigned 1.48% of the overall variation to the component of among group variation. This proportion slightly increased to 1.66%, if commercial varieties were included as seventh population (Table 23).

Table 23: Two-level AMOVA with seven (six populations groups plus commercial varieties) and six (six population groups) populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Without commercial varieties				
Among populations	5	94.472	0.02801	1.48***
Within populations	3710	6939.559	1.87050	98.52
Total	*3715*	*7034.031*	*1.89851*	
With commercial varieties				
Among populations	6	120.751	0.03133	1.66***

Within populations	4143	7681.368	1.85406	98.34
Total	*4149*	*7802.118*	*1.88539*	

d.f. = degrees of freedom. Significance level of the among populations component is indicated by asterisks: *** *p*<0.001.

Similar results were obtained from a three-level AMOVA (Table 24); as sample sizes of commercial varieties were always smaller than ten, this analysis only included the feral populations.

Table 24: Three-level AMOVA with six population groups.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among population groups	5	94.470	0.00199	$0.10^{n.s.}$
Among populations within population groups	29	297.627	0.09212	4.86***
Within populations	3645	6565.500	1.80123	95.03***
Total	*3679*	*6957.596*	*1.89534*	

d.f. = degrees of freedom. Significance levels indicated by asterisks: *** *p*<0.001, *p*>0.05.

Pairwise F_{ST} values were generally low and ranged from 0.0025 (railway stations *versus* oil mills) to 0.0396 (switchyards *versus* road sections), but all except the lowest (railway stations *versus* oil mills) were significant (Table 25).

Table 25: Pairwise F_{ST} values (below diagonal) and their statistical significance (above diagonal).

Population group	BH	FB	GBH	DH	S	OEM	Varieties
BH		0.0078	<0.001	<0.001	<0.001	0.0996	<0.001
FB	0.0048		0.0010	<0.001	<0.001	<0.001	<0.001
GBH	0.0046	0.0063		<0.001	<0.001	0.0010	<0.001
DH	0.0108	0.0231	0.0140		<0.001	<0.001	<0.001
S	0.0199	0.0396	0.0249	0.0085		<0.001	<0.001
OEM	0.0025	0.0076	0.0060	0.0113	0.0238		<0.001
Varieties	0.0154	0.0285	0.0302	0.0227	0.0265	0.0185	

Allelic richness - expressed as expected number of unique alleles - of population groups was lowest in border railway stations and highest in ports and in oil mills (Figure 48). Although allelic richness accumulation curves generally flattened out, only in border railway stations the curve became (nearly) saturated, while in other population groups (especially switchyards, ports, oil mills and also commercial varieties) no saturation was reached (Figure 48).

It appears that especially in ports different OSR varieties are introduced and become established in that area. Additionally, because chemical weed management is not applied here, all of these varieties can persist.

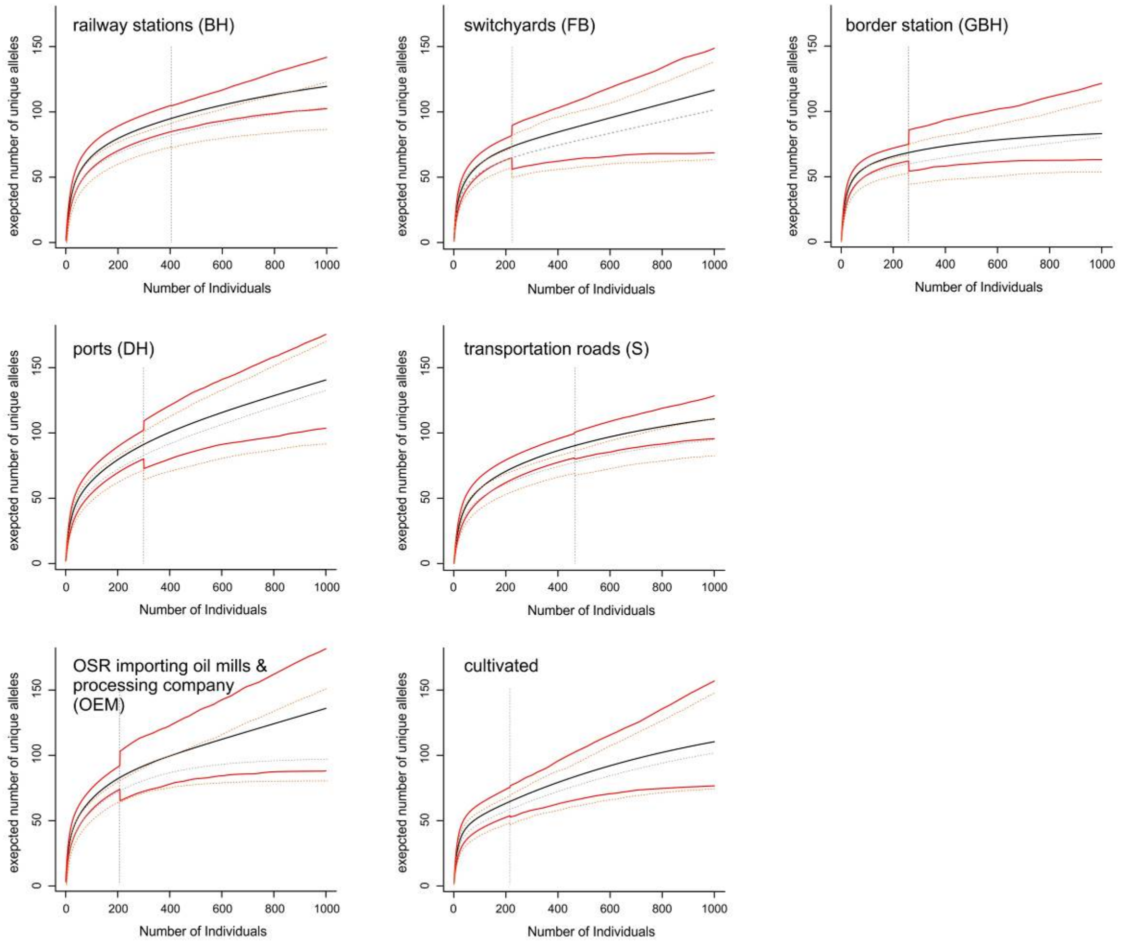


Figure 48: Allelic Richness Accumulation Curves of seven population groups (railway stations, switchyards, border railway station, ports, transportation roads, OSR importing oil mills and processing company, commercial varieties). Mean values (black and grey lines) and their confidence intervals (red and orange lines) are shown for the complete data set (solid lines) and the reduced data set (dashed lines). Vertical dashed lines indicate actual sample sizes.

Applying the DeltaK statistics of EVANNO et al. (2005), *K*=3 was identified as the preferred solution (Figure 49). The resulting genetic assignment is shown in Figure 50. Each of the three gene pools was present in all feral populations. Exceptions were mostly restricted to cases where only few individuals had been sampled (e.g. BHK1), although a few of the larger populations (with 10 or more individuals) contained essentially only two gene pools (e.g. BHK8 or SK03). All three genotypes were present in the entirety of commercial varieties. Within populations, both individuals with no or nearly no admixture were found alongside individuals with genotypes admixed to different degrees, exceptions being small feral populations as well as commercial varieties which usually were genetically relatively uniform (i.e. individuals of the same variety had similar, admixed or not-admixed, genotypic composition).

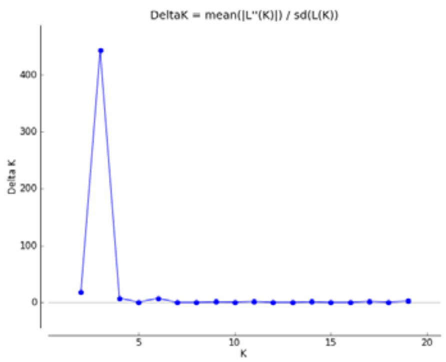


Figure 49: DeltaK values from *K*=2 to *K*=19.

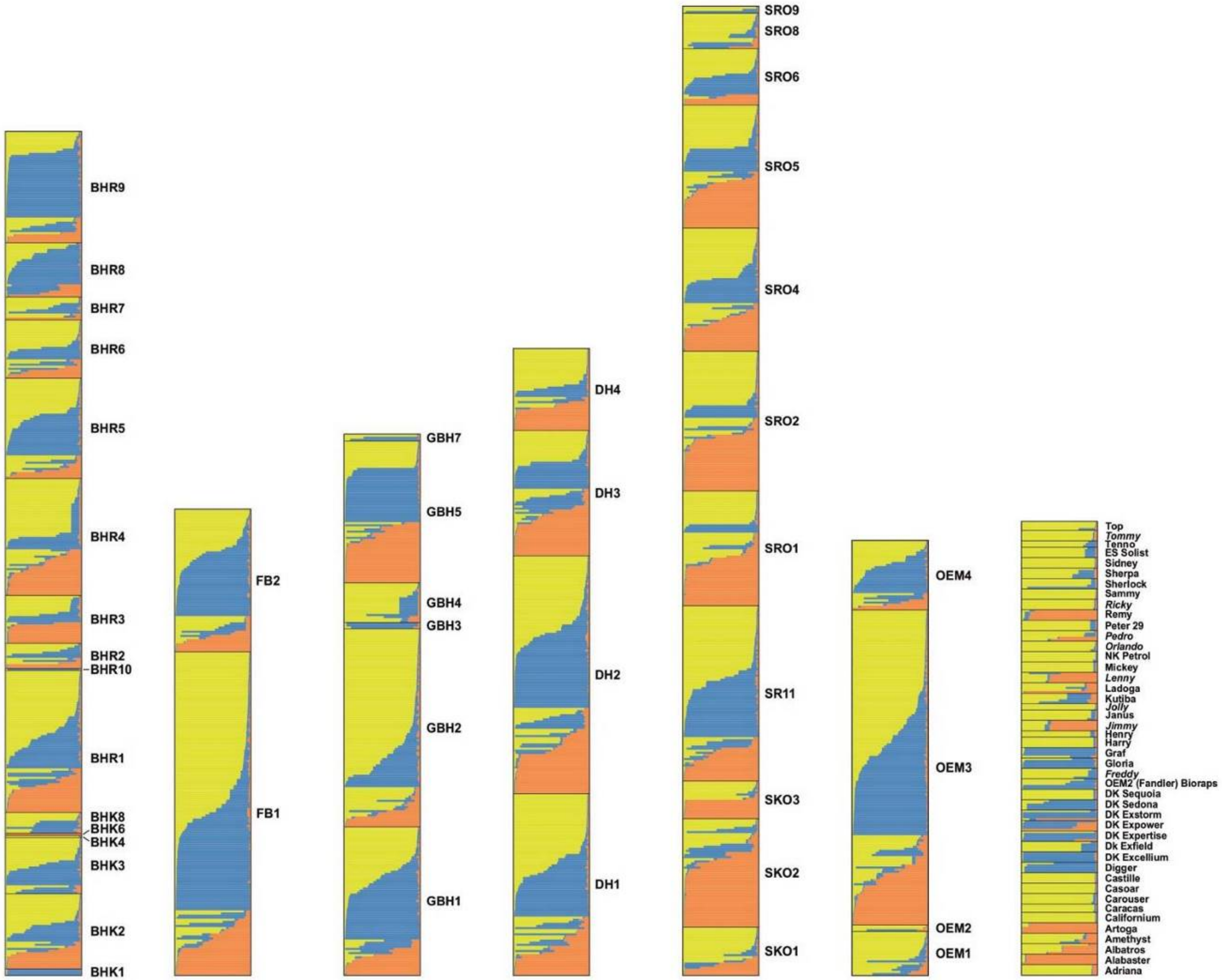


Figure 50: Results of the cluster analysis using STRUCTURE for seven population groups (from top left to bottom right: railway stations [BHK, BHR], switchyards [FB], border railway stations [GBH], ports [DH], transportation roads [SK, SR], OSR importing oil mills and processing company [OEM], commercial varieties). Different colours represent different genetic clusters. Each column represents an individual where the height of the column segments shows the probability of assignment to the respective genetic cluster. Non-Austrian commercial varieties from the EU are indicated in italics.

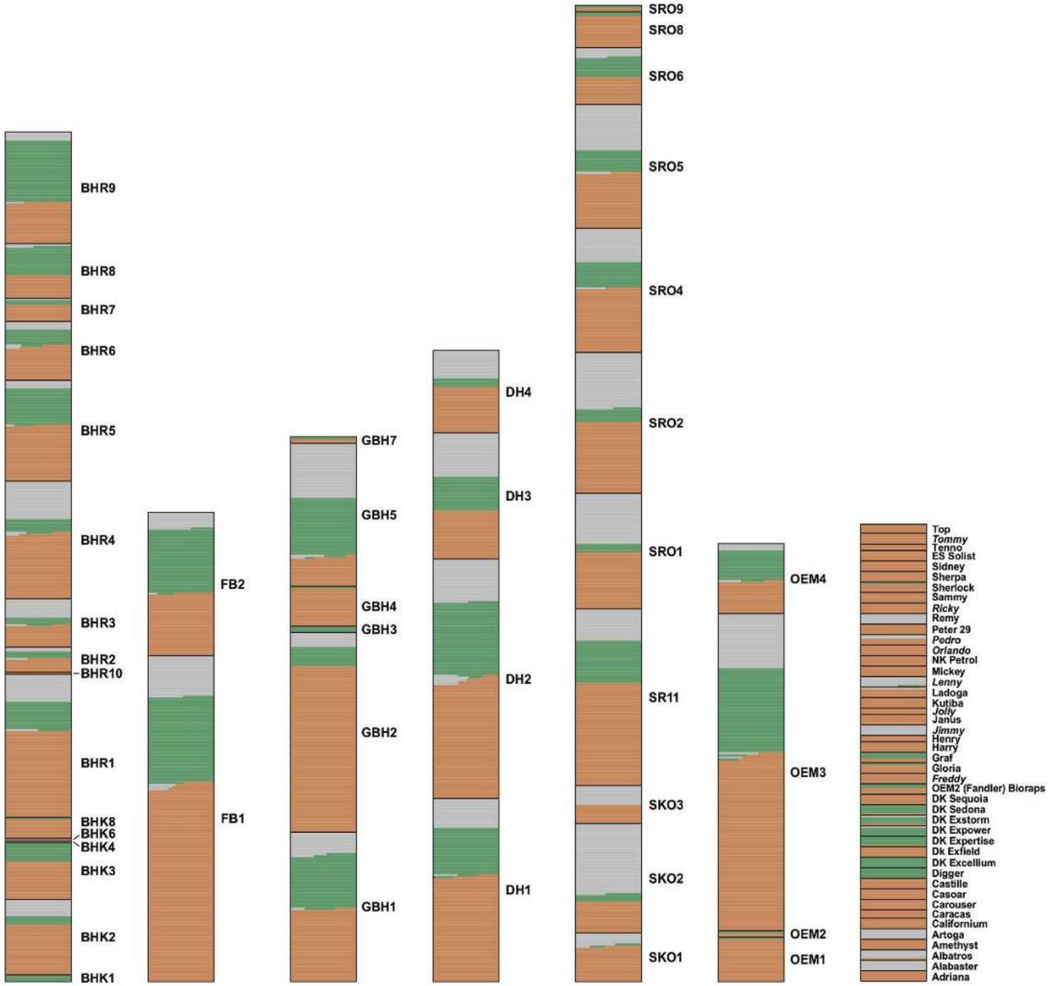


Figure 51: Results of the cluster analysis using BAPS for seven population groups (from top left to bottom right: railway stations [BHK, BHR], switchyards [FB], border railway stations [GBH], ports [DH], transportation roads [SK, SR], OSR importing oil mills and processing company [OEM], commercial varieties). Different colours represent different genetic clusters. Each column represents an individual where the height of the column segments shows the probability of assignment to the respective genetic cluster. Non-Austrian commercial varieties from the EC are indicated in italics.

Clustering results obtained from BAPS with *K*=3 revealed a much lower proportion of admixture compared to the STRUCTURE results, resulting in a much clearer separation of gene pools (Figure 51). Nevertheless in most feral populations each of the three gene pools was present, exceptions again mostly concerning scarcely sampled populations. Irrespective of cluster number (checked for *K*=3 to *K*=10), each identified gene pool was present also in the commercial varieties (although some only in a few individuals), precluding unambiguous identification of foreign gene pools in feral populations.

10. Measures

Measures to limit seed spillage during transport, loading and handling activities which are presently in use and already implemented were checked during fieldwork. For this purpose, inquiries *via* internet and brochures, interviews with managers and loading staff and personal observations directly at the 60 sample sites were made and protocolled.

- The used packing-form during transportation (loose transport, big bags, etc.) is an essential factor for unintended seed spillage. In Table 26 the used mode of transportation system as well as the different forms of packaging which are currently in use are listed. Figure 52 shows two pictures of open loadings of OSR seeds taken at the port of Enns. Especially at ports in storage areas of grain, birds were observed to feed on the grain and could therefore pose an additional distribution factor for OSR grain over longer distances (Figure 52; compare WEDLICH et al. in press).

Table 26: Transportation mode of OSR at sample sites.

Mode of transportation of seeds	*via* trucks	*via* railways	*via* ship		
Institution				Character of OSR	Packing of goods
OEM1: VFI Wels	0%	0%	0%	oil	tanks
OEM2: Fandler Oil Mill	100%	0%	0%	seeds	big bags
OEM3: Bunge Austria GmbH	90%	10%	0%	seeds	?
OEM4: Oil Mill Raab	100%	0%	0%	seeds	big bags
Railways	0%	100%	0%	seeds	goods wagon with unloading hatch
Railways	0%	100%	0%	oil	tanks
Transportation roads	100%	0%	much	seeds	mostly open, big bags
Ports	most	less	most	seeds	loose form

Figure 52: a+b: Ship loading of OSR in loose form at the port of Enns in Upper Austria. **c:** Birds feeding on seeds.

Because of their size of pinheads OSR seeds are frequently lost. This is especially true for open loading forms of OSR, for instance usually used with shipping or transporting on trucks. The loading area of ships and trucks should continually be checked for perishing seals where seeds could get lost. In that case, foam should be inserted in order to darn the holes. Transportation of OSR seeds is safest, when the seeds are packaged in stable big bags.

- Import of OSR seeds by ship was identified to be the main source for accidental introduction of GM OSR seeds to Switzerland (SCHULZE et al. 2014). Hence, testing of grain cargos imported by ship should be intensified to be able to identify GM OSR contamination before unloading, handling and further transportation of the seeds.

- Measures to prevent spillage of imported GM OSR seeds should focus on removal of plants. According to the received information, the Austrian ports, oil mills and OSR processing companies are not treated with chemicals because for these facilities the occurrence of feral OSR plants does not pose a problem. Currently, large plants and populations are manually removed. From an ecological point of view these alternative weed management such as manual removal of plants, mowing or spraying of organic chemicals is welcome and in case, should be intensified. If feral OSR populations would increase or include also GM OSR plants, spraying with chemicals should be considered to unmask GM OSR at that sites and to prevent its unnoticed distribution and persistence.

  Similar to Switzerland, herbicide application along the Austrian railway lines is in the interest of operating companies for economic and safety reasons (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, the gravel of the beds is regularly washed to keep it functional. By this means, the amount of occurring plant seeds in the material is reduced. This is also the reason why gravel beds are renewed. Also additional manual vegetation control measures along railway lines which are already performed in several sampled railway stations in Austria (pers. communication with OEBB staff) should also be increased in agreement with the Swiss recommendations.

- Intense controls of defect wagons coming from abroad should be carried out at the Austrian railway borders to prevent spillage for instance, due to defect or inadequately closed unloading hatches of train wagons.

- Cleaning of loading areas of trucks and ships as well as cargo boards of vehicles and train wagons should be intensified and performed carefully. They should be checked for remaining OSR seeds after cleaning. Also storage areas at ports or oil mills should carefully be cleaned before preserving and storage of the subsequent good.

- If Canadian wheat is imported to Austria in future, the wheat supply shall be tested for GM OSR contamination.

An essential approach would be initiated, if the responsibilities to implement strictly defined measures in order to prevent seed spillage were allocated to the protagonists in the transport system. For instance, the Austrian Ministry for Transport, Innovation and Technology and the Federal Ministry of Science, Research and Economy should predefine mandatory legal guidance (suggestion given by OEBB staff). The main responsibility to limit seed spillage would probably apply to the importers and traders, staff who handles and reloads the seeds as well as seed-processors.

11. Monitoring-program for imported GM OSR seeds and GM feral plants

Identification of commodity flows (“Warenströme”)

If an Austrian monitoring program for imported GM HT OSR was implemented, information about mode of transportation (ship, train, truck), transportation routes as well as loading and handling sites – so called commodity flows (“Warenströme”) – should be obtained to be able to identify relevant sites for monitoring in Austria.

Update of relevant information

Relevant contact addresses (e.g. oil mills, ports, etc.) and sources (e.g. Statistik Austria, INVEKOS, etc.) for necessary information are provided in the present project as supplementary material because of confidentiality reasons. As this basic information is known, the data could be updated immediately, in case of an implementation of such a monitoring. Interviews with the staff of the contacted facilities were particularly helpful to make domestic and foreign goods’ transportation and handling more transparent.

Selection of sample sites and performance of fieldwork

According to the present study, selection of sample sites as well as fieldwork including sampling procedure and survey proved to be appropriate and very useful for a practicable monitoring-program. Recording facts such as sample site description, GPS-data, maturity stage of plants, population size, infestation etc. during sampling are recommended to facilitate interpretation of data. The use of aerial photographs for orientation and for mapping the local positions of OSR-populations is necessary in any case. It is not reasonable to create a general spatial net of equal size to define the area of feral OSR plant survey in all sample sites (Figure 21). Hence, the spatial size of an observation area for feral OSR should be defined site-specific according to the prevailing situation on the spot (e.g. area around railway station, facility ground of an oil mill including receiving roads and tracks in close proximity).

Fieldwork should be performed during the main blossoming season of OSR that means between middle of April to end of June. With the exception of transport roads - except motorways - entering permissions and defined dates to be attended by staff are required for each sample site. Hence, it is recommended that these permits should be organised in time to enable continuous field-work. For sampling along railway tracks as well as in railway stations safety trainings (SIG1 and SIG2) organized by the Austrian Railway Company OEBB had to be attended, in compliance with OEBB safety regulations.

Hotspots of sample sites

If monitoring of (GM) OSR seed spillage during import activities were to be performed under financial and time constraints, it should focus especially on reloading and handling sites of OSR in Austria. In our study, the highest genetic diversity within populations of feral OSR plants was identified for ports and oil mills. This diversity traces back to repeated input of different OSR seeds also coming from abroad. As OSR is received and handled in bulk mixtures complying with quality standards of oil content, absence of GM material and low content of erucic acid and glucosinolates, a broad spectrum of different OSR varieties are present at those sites. If spraying of complementary herbicides was performed in those sites, it would be probable that in case of GM HT OSR imports, feral HT OSR could easily establish. Because the HT plants would have a selection advantage, they could persist over years. Second in line for monitoring turned out to be switchyards which would also be important monitoring sites.

Border railway stations and transportation roads showed the lowest genetic diversity. However, transportation roads as well as railway lines leading to loading, handling and processing facilities would be important for inspection and should be included in a monitoring program (e.g. transportation roads to the Bunge Oil Mill or to the ports of Albern, Krems and Enns).

Method for genetic analyses

Feral plants should be sampled according to the method used in the present study. Analysing around 2,000 individual plants provided comprehensive results. At the same time this sample number is financially feasible. In a monitoring program the largest number of individuals should be sampled at reloading, handling and processing sites because the highest genetic diversity is expected to be found at those sites.

Since single OSR varieties could not be characterised with eight SSR-markers, a larger budget for genetic analyses must be provided to be able to enlarge the SSR-set with additional SSR markers (at least 10 to 20) to improve analysis. Also genetic probes such as Single Nucleotide Polymorphism (SNPs) could be tested for their application to identify single OSR varieties and to assign feral OSR plants to the OSR varieties.

12. Acknowledgement

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- Mr. Wanger from the port of Enns
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