European Network of the Heads of Environment Protection Agencies (EPA Network)
and the European Nature Conservation Agency Heads Network (ENCA) –
Joint EPA ENCA Interest Group on Genetically Modified Organisms (IG GMO)

– Technical Report –

Monitoring of Spontaneous Populations of Genetically Modified Plant Species in the Environment

Experiences and Recommendations for the Design of a Monitoring Programme

Colophon

Prepared for	Joint EPA ENCA Interest Group on Genetically Modified Organisms (IG GMO) Presented at the Oslo EPA Plenary Meeting in February 2019 and at the Vienna ENCA Plenary Meeting in April 2019 to take note of and for information
Supported by	Federal Office for the Environment FOEN (chair), Switzerland Environment Agency Austria Environment & Resources Authority, Malta Federal Agency for Nature Conservation (BfN), Germany Finnish Environment Institute Italian Institute for Environmental Protection and Research ISPRA The Ministry of Environment of Lithuania
Title	Monitoring of Spontaneous Populations of Genetically Modified Plant Species in the Environment - Experiences and Recommendations for the Design of a Monitoring Programme
Date	February 2019
Authors	Federal Office for the Environment FOEN Jan Zünd, Anne Gabrielle Wüst Saucy Federal Agency for Nature Conservation (BfN), Germany Wiebke Züghart Hintermann & Weber AG Christoph Bühler
Contact	Federal Office for the Environment FOEN, Jan Zünd, +41 58 462 20 82, jan.zuend@bafu.admin
Acknowledgments	A project team within the IG GMO has prepared this technical report.

Contents

Preamble

This technical report is published by the Joint EPA ENCA interest group on genetically modified organisms (IG GMO), and provides an overview of the experience with monitoring of spontaneous populations of genetically modified plants (GMPs) in two countries (Switzerland and Germany). The report also provides examples and recommendations for the design of a monitoring programme of GMPs in the environment, based on the experience gathered. The recommendations are for information purposes, and have neither a compulsory nor a legally binding character.

Summary

Aims

The aim of the concepts and methods presented in this report is to allow for the efficient recording in the wild of spontaneous populations of genetically modified plants (GMPs) based on practical experience of GMP monitoring in Switzerland and Germany. This report provides an overview of previous activities to monitor spontaneous populations of GMPs in Europe, and contains recommendations for the planning and design of GMP monitoring programmes.

Experience from these two countries shows that unintended release of GMPs to the environment regularly takes place through import of GMPs for processing or for food and feed. The unintended release, possible spontaneous growth and establishment of GMPs are to be adressed by risk assessors and risk managers. A well-designed monitoring programme that systematically records spontaneous populations of GMPs in the environment is able to generate data, which can be used in the decision-making process and management of GMPs.

Approach

In this technical report, the establishment of a monitoring programme for spontaneous populations of GMPs is presented as a workflow consisting of several different stages. These stages or steps in the workflow are presented and addressed chapter by chapter. The individual stages are described in such a manner that they can be used as an instruction covering all key points. The synthesis of the workflow given on the two following pages provides an overview of the various stages and lists significant steps to be taken when planning and implementing a monitoring programme. These steps will be addressed in more detail in the report. Real-life examples from Switzerland and Germany demonstrate how monitoring programmes for spontaneous populations of GMPs have been put into practice. Each of these examples addresses a different category of location at which GMPs occur. These “monitoring modules” for railway lines, roads, processing facilities or seed and feedstuffs can be implemented independent of each other or in combination.

Key findings

The actual search for spontaneous populations in the wild and the laboratory analysis of plant material are only the final steps in a chain of actions. For the monitoring programme to be efficient and meaningful, preliminary work must be undertaken. This work will ensure that surveys are limited to plant species and locations that are relevant, i.e. those which can reasonably be assumed to give rise to a higher probability of spontaneous populations of GMPs from an unintended release of the GMP to the environment.

Relevant GMPs and contaminated goods
As a first step, plant species must be identified which should generally be taken into consideration for a monitoring programme, i.e. those GMPs that are able to grow spontaneously under European climatic conditions. Hypothetically, a single grow-out could be enough to introduce the transgene into the environment. Once the GMPs potentially capable of growing are known, the most likely entry pathways into the environment and release locations, through which unintended release of the GMPs occur, need to be identified. An analysis of the flow of goods entering a given country is useful in this respect. It identifies the goods that may be contaminated with GMPs capable of growing and which may enter the area to be monitored. Crucial factors to be taken into consideration include whether the target GM crop plant is cultivated in the country of origin, the imported quantities, the goods‘ processing status and their packaging, and whether the goods are customarily introduced into the environment in line with their intended use (seed, feedstuff, straw, decorative material, fireworks, and others).

Release locations
Once the relevant GMPs and potentially contaminated goods are known, the locations need to be identified for which the goods are destined and where the undesired release of GMPs is likely to occur. What is needed is a very specific list or map of these locations. The desired list can be compiled from searches in the commercial register, enquiries with industry associations and selected companies, internet searches or by conducting an analysis of customs authority data. On this basis it is possible to deduce the survey area which ultimately is to be surveyed for spontaneous populations of GMPs.

Survey objectives and strategy
The now identified survey area is often too large for a complete survey or full-coverage search. It will be necessary to take a random sample of locations that allows for statements to be made on spontaneous populations of GMPs. Depending on the type of statement to be made, the random sample is defined on the basis of one of three strategies: a) a purely random sample, b) a random sample with a higher emphasis placed on locations characterised by a known maximum probability of the presence of GMPs, or c) a random sample giving special consideration to locations for which there is as yet little knowledge as to the probability of the presence of GMPs. For strategies b) and c) the random sample can be optimised on foot of each survey round by promptly incorporating the knowledge gained (adaptive design). If the locations with a higher than normal probability of the presence of GMPs share common characteristics, such an adaptive sampling design significantly improves the number of confirmed GMPs or the accuracy of the estimate for the entire population of GMPs.

Fieldwork and laboratory work

Once the objectives and the survey design for the GMP monitoring programme have been established it can be implemented. Suitable methods are required for the search for spontaneous populations of GMPs in the wild as well as for the laboratory analysis of collected plant material. It is important that both of these steps in the workflow are conducted in a precisely defined and replicable manner. In Germany and Switzerland, published proven standards are available to this end for both field and laboratory methods.

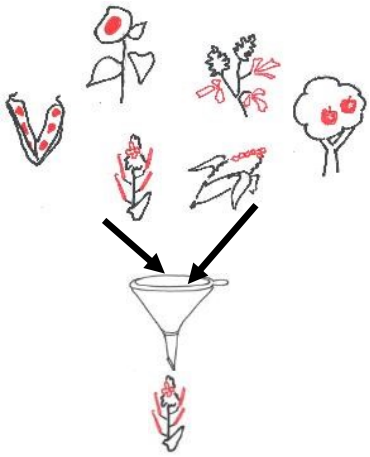
Overview: The pathway to monitoring spontaneous populations of GMPs

For the steps denoted with an asterisk* this report provides specific instructions or aid.

Starting point for GMP monitoring
Where monitoring is a component of import approval, the current legal situation must first be clarified.

Identification of relevant GMPs

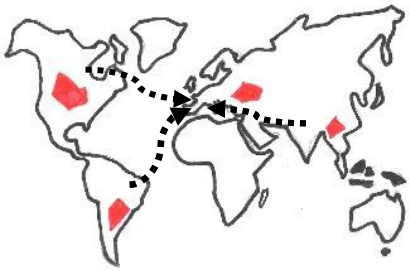
Which GMPs are on the market?
Which of these could grow spontaneously in the area?
Which of these could enter the area?
Which of these must primarily be monitored?



→ *Step 1: Assessment of biological characteristics — Ch. 3.2
→ *Step 2: Appraisal of probability of being imported — Ch. 3.2
→ *Step 3: Priorities based on GMPs' risk potential — Ch. 3.3

Identification of relevant imported products

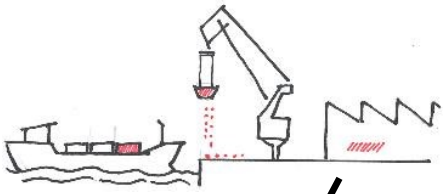
Which of the imported goods could contain GMPs identified as being relevant?
In what quantities are they imported and who are the recipients?



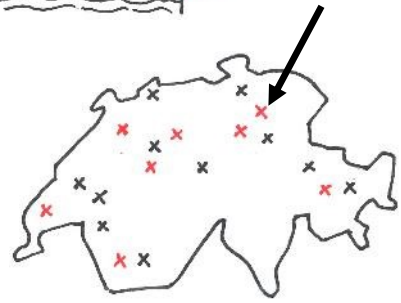
→ *Analysis of origins and flow of goods in the area — Ch. 3.4, Ch. 4.4

Narrowing down release locations

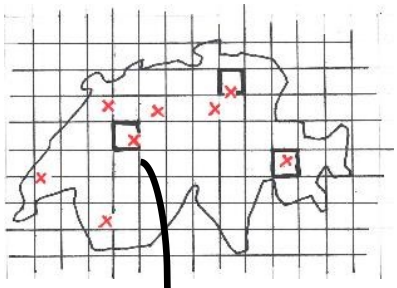
What are the exposure pathways?
At which locations could GMPs be released?



→ *Clarifications re modes of transport, packaging, and recipients of goods — Ch. 3.4
→ *Drawing up of lists and maps of locations of recipients and access routes — Ch. 4.4



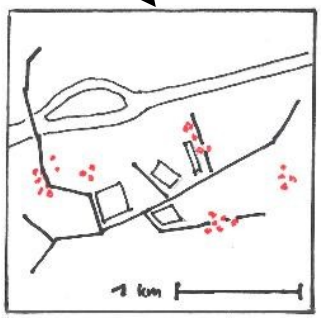
Drafting the monitoring strategy



What is the monitoring to achieve?
In what timeframe are results needed?

→ *Establishing the survey area Ch. 4,5,7
→ *Clear definition of the monitoring programme's objectives Ch. 4,5,7
→ *Get inspiration from existing projects Ch. 4 &5

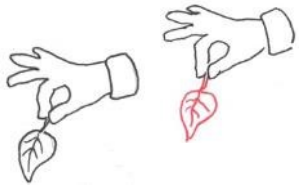
Planning the survey



Is area search possible or is random sampling required?
What needs to be acheived? What is the aim?

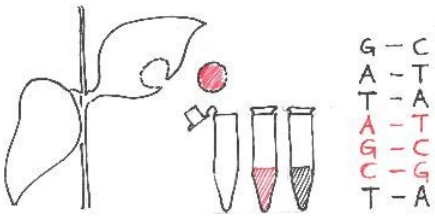
→ *Choosing strategy for random sampling design
→ *Definition of indicators / metrics Ch. 7
→ Drafting the data analysis process Ch. 7

Establishing methods to be used

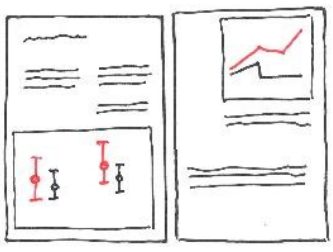


What detection methods are available?
How should fieldwork be conducted?
What are the costs for sampling and analysis?

→ Specific clarifications with candidate laboratories
→ *Draw up instructions for field survey Ch. 6
→ *Determine standard operating procedures for sampling and analysis Ch. 6



Conducting the surveys and survey analysis



Are there already any indications of common characteristics shared by locations at which GMPs occur spontaneously?
Based on these findings, could subsequent surveys be conducted in a more targeted manner?

→ (Statistical) data analysis
→ *Taking results into consideration for planning the next survey (adaptive sampling) Ch. 7

1 Context

1.1 Rationale

The ability to recognise spontaneous populations of GMPs in the wild is a key component of comprehensive GMP monitoring. It is the prerequisite to being able to investigate potential impacts on the environment of spontaneously released GMPs.

The actual frequency of GMP populations and their geographical locations has been studied in different countries, albeit mostly in time-limited case studies (Greene et al. 2015; Saji et al. 2005; Knispel & McLachlan 2010; Lee et al. 2010; Schafer et al. 2011). However, clear guidelines for the methodology to be employed for long-term monitoring (e.g. Katsuta et al. 2015) are lacking. This is a gap the present report aims to fill. It is based on concepts developed in Switzerland and Germany to date. Drawing on the experience gained in these two countries, the report presents recommendations that are as universally applicable as possible and makes these accessible to interested parties. Supplementary content was obtained from a review of the scientific literature or was newly developed (e.g. the notes on survey design in Chapter 7).

1.2 Aims and objectives

This technical report provides methodological guidelines for designing a monitoring programme for spontaneous populations of GMPs. The aim of the concepts presented here is to allow for the detection of GMP populations in the wild in as efficiently a manner as possible. The technical report serves as a best-practice aid and as an instruction for the establishment of such a monitoring programme. Its target audience includes authorities, applicants, and all other persons faced with the task of conducting GMP monitoring. They are provided with clearly set out instructions which guide them step-by-step through the individual decisions to be taken and work to be conducted while taking into consideration all essential aspects.

The present report is a purely technical contribution. It provides recommendations and serves as a reference for all user groups involved in monitoring spontaneous populations of GMPs. The question as to whether it is sufficient to follow these recommendations to meet the legal requirements of GMP monitoring, as applicable, must be addressed by the users themselves.

1.3 Which type of GMP monitoring?

Genetically modified organisms (GMOs) have been the subject of monitoring programmes for more than 30 years. Such programmes can be targeted at very different aspects. They can, for example, monitor the presence of transgenes in foods or observe the impacts of cultivating genetically modified (crop) plants (GMPs) on non-target organisms. GMO monitoring within the meaning of the

present report is primarily limited to the detection of undesirable populations of GMPs in the wild. It provides an efficient mechanism for the potential detection of GMPs in locations at which they should not or must not grow. In addition, it also addresses the detection of GMPs in goods contaminated with them, but only with respect to those types of goods the use of which could result in GMPs being released into and spontaneously growing in the environment.

This report does not address the general surveillance of goods for the presence of GMPs with a view to verifying that the goods are correctly labelled or meet particular quality parameters. Equally, the report deliberately excludes concepts designed to document the impacts of GMPs on humans and the environment.

Therefore the starting point for a monitoring programme within the meaning of this report is not the cultivation of GMPs but their importation and the processing of goods containing GMPs. These include either

- approved imported products containing GMPs with the products being destined for sale or processing (but not as seed) or
- goods illegally containing GMPs, i.e. goods that are labelled incorrectly or goods containing inadvertent admixtures of GMPs.

1.4 Regulatory framework

GMP monitoring can be subject to a range of different legal requirements. Import approvals or operational permits may impose a duty to monitor on the permit holder, or authorities may want to engage in environmental monitoring of GMPs.

In Switzerland, the handling of genetically modified organisms is regulated by the Gene Technology Act (GTA[1]) and its related ordinances. With respect to monitoring, the licence application for placing genetically modified organisms on the market must contain a monitoring plan (Article 28 Release Ordinance [RO] [2]). The competent authority has the right to demand further investigations in addition to the monitoring plan (Article 44(2) RO). Moreover, the GTA and RO require the Federation to establish a monitoring system (Article 24*a* GTA and Article 51 RO).

At the European level, Directive 2001/18/EC[3] regulates the release of genetically modified organisms. It also includes provisions for GMP monitoring which must be transposed into national law in the individual EU Member States (e.g. Article 20).

1 SR 814.91.

2 SR 814.911.

3 Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L 106, 17.4.2001, p. 1–39). **Environmental monitoring** is a means to verify assumptions and conclusions drawn in the environmental risk assessment and to identify possible effects of GMOs, which were not anticipated in the ERA. According to Directive 2001/18/EC and Council Decision 2002/811/EC2, monitoring plans have to be implemented in order to trace and identify any direct or indirect, immediate, delayed or unforeseen effects on human health and the environment of GMOs or its use after they have been released into the environment. The Annex VII of Directive 2001/18/EC describes the objective of the monitoring in more detail: […] “to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the ERA are correct” (case specific monitoring) and “to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the ERA” (general surveillance).

In order to assess the specific legal situation, it will therefore be necessary to consult the regulatory framework in the Member State concerned.

In Germany, the duty to monitor approved GMOs was transposed into German law with Article 16c of the Genetic Technology Act (GenTG[4]). It places on the distributor of GMO products the duty to monitor the GMOs in accordance with the licence granted in order to detect potential impacts, e.g. impacts on the environment.

1.5 Key to success

The detection of spontaneous populations of GMPs is the endpoint of an entire cascade of separate steps, with each step being dependent on the previous one. The detection of spontaneous populations of GMPs can only be successful if all the steps have been completed.

- Survey area: It must be delimited in such a way that it definitely covers potential spontaneous populations of GMPs.
- Probability of detection: The manner in which field mapping is conducted must ensure that the locations hosting GMPs are indeed visited (e.g. correct timing, duration and survey route).
- Sampling success: Field sampling must be sufficiently detailed to effectively include the transgenic plants.
- Probability of detection in the laboratory: Laboratory methods must be sufficiently reliable so as to ensure that a transgene contained in a sample will be detected with very high reliability.

All these steps are of equal importance and must be carefully planned. The present report serves as a working aid designed to contribute to this process.

1.6 Terms

This section sets out the meaning of some terms that are used repeatedly in this report:

- Exposure (of GMPs): Uncontrolled population of living GMPs in the wild, allowing for interactions between the GMPs with the environment and wild organisms.
- Exposure pathway: The pathway along which plants or components thereof (e.g. seeds, pollen) are transported before they are released into the environment.
- Release location (of GMPs): End of the exposure pathway. Location at which the GMPs enter the environment and where they may grow spontaneously (e.g. the location where spilled seeds of GMPs germinate).
- Contamination (with GMPs): Contamination of a product with GMPs, i.e. admixture of GMPs to another product. This term does not differentiate between intentional and unintentional contamination, nor does it contain a statement as to whether the level of contamination is above or below an approved threshold value, as applicable.

[4] https://www.gesetze-im-internet.de/gentg/__16c.html

- Risk: Possibility of damage occurring as a result of a certain event or behaviour. Risks refer to hazardous situations which may or may not result in adverse impacts. Expressed mathematically: Risk = probability of occurrence x extent of damage.
- Spontaneous plants: Plant specimen(s), which grow spontaneously at least once under current climatic conditions. These do not necessarily need to flower or set seed as part of their growth. This term does not express whether or not the plants will become established (become feral) in the long term.
- Spontaneous population: Spontaneous population with the plant(s) being able to flower or set seed at least once or to vegetatively reproduce.
- Becoming feral (GMPs): Synonym of becoming established. Continued existence of a GMP in a natural or semi-natural biocoenosis of plants (which exists uncontrolled in the wild). The plants are able to reproduce autonomously or at least persist vegetatively. In any other case we speak merely of spontaneous populations.

2 Impacts of spontaneous populations of GMPs in the environment

Where GMPs occur spontaneously in the environment, this may give reason for monitoring these occurrences. It is possible for these spontaneously growing GMPs to adversely impact the environment or to cause economic damage. Moreover, depending on the specific country or situation, spontaneous populations of GMP may be illegal or undesirable. The undesirable release of GMPs, including the release outside of cropland, has been documented repeatedly (Section 2.1). While it is plausible that this may have harmful impacts, conclusive evidence to this effect is still outstanding. However, there has as yet been little research on the presence and impacts of spontaneously released GMPs in the wild and therefore robust factual evidence is hard to come by (Section 2.2).

A recent review by Tsatsakis et al. (2017) discussed potential direct and indirect impacts of GMPs on the environment. Many of the scenarios set out in the paper have not yet occurred in the roughly 20-year history of GM cropping, or they have not yet been substantiated sufficiently. Some of the potential impacts not only concern the commercial cultivation of GMPs but may also result from spontaneous populations of unintentionally released GMPs. In particular, these include

- the outcrossing of transgenes into GM-free crop plants or into related wild plant species, and
- the uncontrolled or even invasive spread of GMPs.

2.1 Experience to date

Undesirable populations or transgene outcrossing to crop wild relatives have already been observed for at least eight different GMPs[5] (Bauer-Panskus et al.

[5] Genetically modified maize, rapeseed, soya, cotton, rice, alfalfa, papaya and poplars.

2013 and 2015). It is not always evident how these events occurred, nor to what extent seeds or pollen were responsible. In some cases undesirable releases as a result of transport losses play a significant role (see below).

It is notable that in almost all of the cases of spontaneous GMP populations that have become known, the plants in question are designed to be herbicide tolerant. While herbicide tolerance is the trait most frequently used to modify GMPs, it is also particularly easy to recognise in the field. These plants are very noticeable following herbicide applications as part of the management of railway lines or company premises. Cases involving other frequently used genetically engineered traits, such as Bt transgenes, have been reported much more rarely (e.g. Dyer et al. 2009). They would be much harder to recognise and have hardly been studied to date. For many developing countries and newly industrialising countries no published studies are available at all on spontaneous populations of GMPs, for example on spontaneously growing GM soya in South America.

However, the following examples outline proven cases of undesirable populations of GMPs; transport losses are at least one of the exposure pathways involved in these cases:

- In Japan, spontaneously growing GM oilseed rape plants were found in 80% of seaports surveyed (Katsuta et al. 2015). Apparently the plants originated from seeds spilled in the course of unloading imported goods. A similar picture emerged in South Korea, where additionally occasional populations of GM maize and GM soya plants were recorded (Bauer-Panskus et al. 2015). GMPs are cultivated neither in Japan nor in South Korea. GM oilseed rape plants have also been found at the Rhine Port in Basel, Switzerland (Schulze et al. 2014). It is highly probable that these plants' origin was contaminated Canadian durum wheat, which is transported to Basel by ship and then transferred to silos and railway wagons for onward transport (Schulze et al. 2015). This Swiss study found the three different GM oilseed rape events GT73, RF3 and MS8×RF3 in spontaneously growing oilseed rape plants. In addition, the study found outcrosses of GT73 oilseed rape plants to two non-GM oilseed rape plants. However, transgene outcrossing to related wild species was not detected.
- Spontaneous populations of GM oilseed rape were found along railway lines in Canada and Switzerland (Yoshimura et al. 2006, Schönenberger & D'Andrea 2012). These are likely due to transport losses. In Mexico, railway accidents resulted in unground Bt cottonseed and Bt maize being spilled. From 2010 to 2013 alone, losses of approximately 800 tons of transgenic maize and transgenic cotton were recorded. It is assumed that these losses have contributed to the spread of the transgenes of these two GMP species (Piñeyro-Nelson et al. 2009; Dyer et al. 2009, Bauer-Panskus et al. 2015).
- Spontaneous populations of GM oilseed rape have been documented for the Canadian oilseed rape-growing region (Knispel et al. 2008). At least the GM oilseed rape plants growing along roadsides originate from agricultural transports of harvested crops. Glyphosate resistance was found in 88% of these roadside populations and approximately half of the populations were resistant to two active ingredients. The undesirable release of GM oilseed rape along roadsides has also been documented in the United States (Schafer et al. 2011) and Australia (Busi & Powles 2016) and are likely due to spillage during transport.

- Undesirable populations of GM herbicide tolerant alfalfa along roadsides have been documented in major alfalfa seed-production areas in the US (Greene et al. 2015). These spontaneous populations have been ascribed i.a. to losses during transportation of harvested crops to processing facilities. Depending on the area, the plants occurred at a frequency of between 1 and 8 plants per 100 km of road. The frequency of sites having transgenic alfalfa plants varied between 8.3 and 32.7% in the different study areas. Outcrossing of alfalfa to wild species is unlikely in North America as the continent does not host any close relatives of alfalfa.

2.2 Potential impacts

Outcrossing
If there are related crop species or crop wild relatives in the vicinity of spontaneous GMP populations, GM pollen may be transferred and passed on to hybrid plants.

The probability of a transgene outcrossing to GM-free crop plants or wild plants is dependent on the environment into which the GMP is released (Tsatsakis et al. 2017):
- Agricultural areas with GM crop cultivation,
- Agricultural areas without GM crop cultivation,
- Non-agricultural areas.

The quantities of GM pollen produced, the densities of donor plants and recipient plants, the presence and abundance of pollinators, and the prevailing abiotic factors differ between these different types of environments. There has as yet been little research on how exactly these factors impact on the probability of outcrossing. However, it is reasonable to assume that the outcrossing rate of spontaneous populations of GMPs outside of areas in which GM crops are cultivated differs from that of GM crops.

Hybridisation between GMPs and wild plant species has to date primarily been studied in agricultural cropping situations. Tsatsakis et al. (2017) provide an overview of the findings. These situations are typically characterised by high densities of GM donor plants, while the density and diversity of pollinators and wild recipient species tend to be rather low, not least owing to intensive pesticide use. The situation may be different for spontaneous populations of GMPs outside of GM cropping areas; it is possible that in these areas a significantly greater amount of cultivated and wild recipient species is “available” and the probability of outcrossing may be greater even though the low number of spontaneously growing GM specimens produce a relatively small quantity of pollen overall.

Persistence and invasive behaviour
Expert opinion differs on whether transgenic crop plants may indeed develop into invasive weeds (Tsatsakis et al. 2017). Whether or not this will happen depends on the environment in which the GMPs grow spontaneously, or rather on whether the specific traits confer on them a fitness advantage in a given environment. Only a small number of studies have compared the fitness of hybrids between GMPs and conventional cultivars or crop wild relatives. Individual studies have shown that persistence or invasive behaviour are not beyond the realms of possibility. In

the case of soybeans, Guan et al. 2015 found that the hybrids did not suffer a competitive disadvantage compared to the wild soybean (*Glycine soja)*, even in the absence of herbicide applications. In sunflowers, Mercer et al. (2007, 2014) found increased or only slightly decreased fitness in crop-wild hybrid genotypes if these were subject to competition and herbicide applications. In addition to herbicide tolerance, traits such as increased growth rates, stress tolerance or the ability to self-pollinate may favour the evolution of problem weeds in agricultural cultivation (Tsatsakis et al. 2017).

Creeping bentgrass (*Agrostis stolonifera*) is a well-known example of the ability of GMPs to become established as weeds and form persistent populations. In Oregon (USA), genetically modified, herbicide tolerant creeping bentgrass was released on a trial basis in 2002. A storm caused widespread dispersal of transgenic creeping bentgrass pollen. Outcrossings to local bentgrass populations were found even at distances of more than 20 kilometres (Watrud et al. 2004). The transgene also introgressed into the closely related perennial grass *Agrostis gigantea* and even transgenic intergeneric hybrids have been found (Zapiola & Mallory-Smith 2012). All attempts at eradicating the transgenic plants and hybrids have failed so far and the company responsible had to pay a fine of $0.5 million for non-compliance with conditions. Transgenic creeping bentgrass can now be found on roadside verges, in irrigation channels, in grassland and on arable land (Snow 2012).

Fig. 1: Urban environment containing various types of infrastructure. Residual areas of various sizes that are not regularly used or not used in a targeted manner can be found in between the infrastructure installations (photo: Thomas Stalling).

Potential damage

The unintended release of GMPs into the environment does not inevitably result in ecological or economic damage. However, if a GMP or its transgenes continue

to spread, consequential damage may well result. Experts have investigated and discussed at length concrete scenarios of damage caused by the unintended release of GMPs (see the review by Nicolia et al. 2014), always focusing however on commercial cultivation as its cause. Of relevance to humans are both damage caused to nature and the environment and the resultant impacts on the national economy. While severe effects on the environment have not as yet been observed, some of the scenarios have come to pass, at least in part.

- **Adverse impacts on protected habitat types:** If in the vicinity of habitats of conservation concern GMPs spread uncontrolled due to fitness advantages, conservation objectives may be hindered (Lang et al. 2015, Menzel et al. 2005). Rare or endangered habitat types could be altered in their specific manifestation and species composition. GM alfalfa for example harbours potential for such a development. Feral alfalfa plants were discovered at more than 400 sites along roadsides in alfalfa seed-production areas in the United States (Greene et al. 2015). GM-free cultivars of this crop plant tend to be invasive in central European dryland habitats.
- **Increased labour intensity:** This scenario is similarly based on potential invasive behaviour of GMPs. The amount of labour required to maintain roadside verges and railway lines would likely be higher in the presence of herbicide tolerant plants (Devos et al. 2012). In the case of creeping bentgrass, the introgression of the transgene into wild populations constitutes a first step towards the development of a “super weed” (Snow 2012).
- **Income foregone**: Contamination with transgenes of goods declared as GM-free results in economic damage, be that in cultivation or in the processing of agricultural products. For example, in 2006 a GM rice not approved for cultivation was mixed with long grain rice and exported to more than 30 countries around the world. According to an estimate by Greenpeace, losses of up to $253 million resulted from food-product recalls in Europe alone[6].

3 GMPs in the environment

This chapter describes the preliminary steps that are essential for the targeted monitoring of undesirable populations of GMPs.

- Firstly, this concerns criteria to identify those GMPs which should be prioritised for monitoring (Section 3.1 and 3.2).
- Secondly, this concerns the search for goods which may be contaminated with GMPs. This is important because spontaneous populations of GMPs can often be traced back to transport losses of goods containing GMPs. Moreover, this involves the identification of pathways through which these GMPs are unintentionally released to the environment (Section 3.3).

Step-by-step these clarifications allow for the identification of relevant GMPs, contaminated goods, their exposure pathways and release locations (Fig. 2). Once these factors have been isolated, a suitably targeted monitoring programme can be planned and established. Tangible examples of practice- oriented monitoring modules are described in Chapter 4.

[6] www.reuters.com/article/idUSIndia-30351820071106

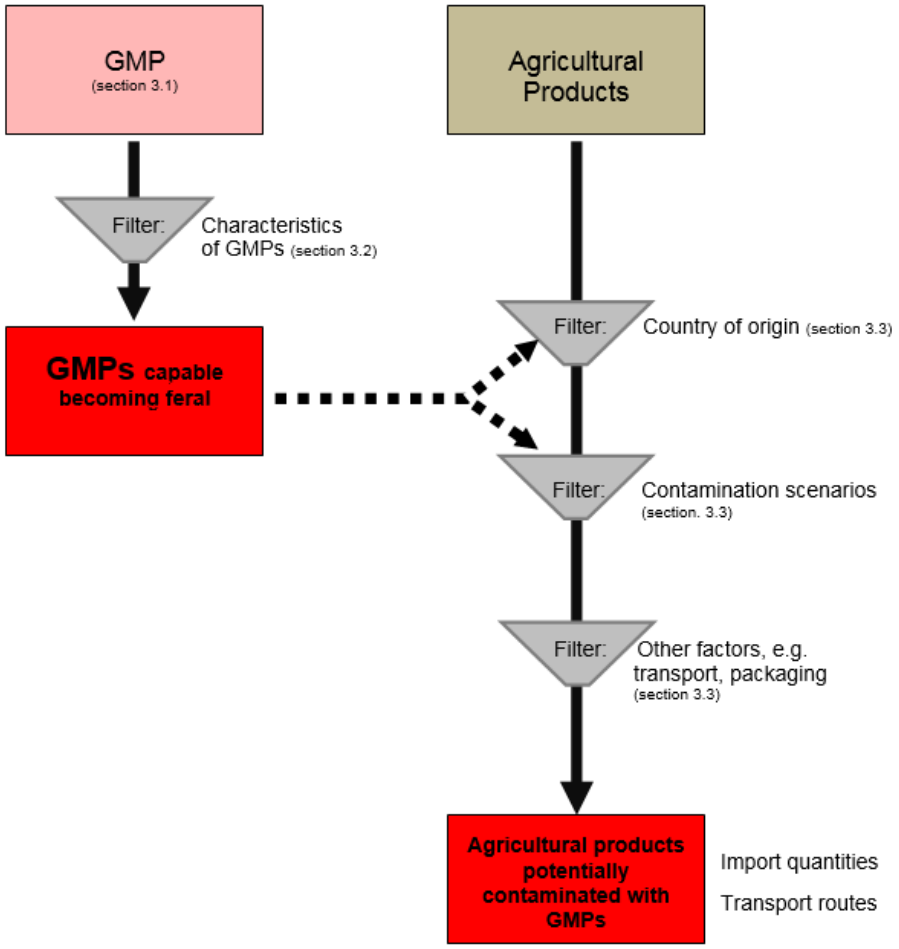


Fig. 2: Diagram outlining the procedure for identifying relevant genetically modified plants and the goods which may contain them. The necessary steps to this end are detailed in different sections of Chapter 3.

3.1 Which of the GMPs are relevant?

The aim of the monitoring described in this report is to detect GMPs that spontaneously and unintendedly grow in the wild. But which of the spontaneous populations are relevant for a monitoring programme?

- If the aim is to assess the efficiency of management measures taken, e.g. to prevent spillage during transportation of GM seed capable of germinating, initially all spontaneous populations of GMPs are of equal significance. The steps set out in Section 3.2 are sufficient to identify the GMPs relevant for the monitoring programme.
- If the aim is to identify those GMPs that are more likely to result in adverse environmental impacts, a more detailed assessment is required. In this case, the GMPs are those that harbour a particularly high potential of becoming feral or causing damage. In order to identify GMPs of relevance to the

monitoring programme, the clarifications pursuant to Section 3.2 must be complemented with the process of prioritisation as set out in Section 3.3.

3.2 GMPs capable of spontaneous growth

For monitoring it is immaterial whether spontaneously occurring GMPs are plants that only grow out once and subsequently vanish or plants which establish persistent populations. What is crucial is the fact that as a result of the GMPs' growth transgenes are exposed to the environment, even if a GMP successfully grows only once and, for example, produces pollen which is transferred to wild relatives by wind or insects. Therefore, there is a need to monitor spontaneous populations if the following condition is met:
Starting from plant parts distributed in the wild, a GMP is able to grow spontaneously in the survey area under current climatic conditions and either

a) flower and/or set seed at least once, or
b) vegetatively reproduce.

We term GMP species meeting this condition GMPs "capable of spontaneous growth". The GMPs which are capable of spontaneous growth can be determined in two steps[7].

Step 1: GMPs with suitable abiotic conditions
GMPs have mostly been bred from existing crop plants. For these crop plants (i.e. the non-transgenic cultivars), solid information tends to be available that allows for an assessment as to whether there is a likelihood of spontaneous populations to occur or not. This information needs to be researched. To this end a range of different information sources are available:

- Documented records of spontaneous populations in floras, botanical journals, or floristic databases. If a species is listed in any of these publications it can be assumed that it will be able to survive under local climatic conditions. However, often it is not clear whether the plants in question belong to persistent established populations or to transient populations which keep appearing only to vanish soon after. Examples of the latter are tomato and pumpkin plants on riverbanks and in central European floodplains.
- Publications on cultivation, persistence and volunteering in the agronomic literature. An Internet search, e.g. using "Google Scholar", quickly provides a great number of indications. The fee-based "Web of Science" citation indexing service allows for comprehensive and highly targeted access to journal articles. Useful search terms on growth behaviour include words such as "feral, spontaneous, adventitious, volunteer, weedy". Searches of this kind can indeed turn up results which run counter to spontaneous appraisals, as is the case, for example, for maize in Austria (Pascher 2016) (Fig. 3) and wheat in Switzerland (Kalinina et al. 2015).
- Assessments by specialists: Staff at agricultural research centres or research institutes know a lot more about their research subjects than what can be

[7] It is important to bear in mind that the review given above merely describes the current situation involving currently existing GM plants. Both the development of new GM plants and climate change may alter this situation in the medium term (see the "Outlook" Chapter).

quickly accessed in publications. For example, it would be very difficult to conclusively answer the question of whether or not the soybean could become feral in central Europe without expert knowledge on the climatic suitability of currently available cultivars.

Figure 3: Spontaneously growing maize at different sites in Austria (Photos from Pascher, 2016).

These information sources provide solid indications of the growth behaviour of GMPs. However, this is merely an assessment of whether not a GMP could occur spontaneously. Even if the answer is “yes” it is a question of probability whether or not it thrives in the wild. Among other factors, this probability depends on the type and quantity of plant components entering the environment in the first place, and on the degree to which site conditions occur in the wild that are necessary for the plant to thrive. Spontaneous tomato populations for example tend to be merely occasional and subpopulations are largely limited to well hydrated and nutrient-rich sites, e.g. alongside watercourses or composting facilities in urban areas. In contrast, suitable site conditions for oilseed rape are much more frequent in the entire agricultural area. Any kind of sparsely vegetated site will suffice. Section 3.2 will therefore discuss more detailed assessments of the actual probability of the spontaneous growth of GMPs potentially capable of becoming feral.

Step 2: GMPs that are highly likely to be imported

The list of GMPs capable of spontaneous growth compiled in Step 1 can be of considerable length, especially for countries enjoying a warm-temperate climate or mild winters. Additional criteria can be used to further narrow down this list in a second step. The criteria used to this end assess the actual probability of individual GMPs entering a (survey) area. They take into consideration the flow of goods, transport routes used, and also natural processes contributing to the spread of plant components. Basically the same considerations are applicable here as in Section 3.4 which assesses whether or not imported goods may be

contaminated with GMPs. That section also describes in greater detail the type of research required to answer the following questions:

- Is the GMP approved for importation, processing, food or feed?
- Is the GMP used in cultivation trials in the survey area?
- Are there significant imports (roughly 100 tons or more) of agricultural products from countries in which the GMP is cultivated?
- Are there imports of agricultural products from countries in which the GMP is cultivated that are introduced into the environment in a targeted manner (seed, hay, straw, feedstuffs, ornamental plant material)? In such cases, imported quantities of less than 100 tons are also of significance.
- Is the GMP present in directly adjacent countries/regions that are connected to the survey area through major distribution axes such as railway lines, roads or watercourses?

If for a specific GMP at least one of these questions is answered in the affirmative, the GMP will clearly remain on the list of potential candidates for GMO monitoring. If all questions are answered in the negative, the GMP in question is currently not of significance for a monitoring programme, despite its existing propensity for occurring spontaneously.

Intermediate result
The two steps described above have now resulted in the list of potential candidate GMPs for monitoring: They comprise those GMPs which are able to a) grow spontaneously and b) effectively enter the survey area.

The results of these clarifications may already be reason enough to search for undesirable populations of GMPs. It may be necessary to further narrow down the resultant list of GMPs, e.g. if the focus is strictly on GMPs with higher damage potential or if limited funding necessitates a stricter selection. The steps outlined in the following Section 3.3 will therefore describe additional priorities for the selection of GMPs to be monitored.

3.3 Which GMPs should be given priority?

Step 3: Is prioritisation necessary?
The initial clarifications have resulted in a list of those GMPs that are capable of spontaneously growing in the survey area (Steps 1 and 2 in Section 3.2). If it is necessary to further narrow down this list, the primary criterion should be the individual urgency of monitoring specific GMPs. The practical feasibility of monitoring could be an additional selection criterion. However, we are exclusively concerned here with the urgency to monitor as seen from a technical perspective. The urgency to monitor spontaneous populations of specific GMPs increases with the extent and likelihood of damage associated with the spontaneous population, spread, establishment and outcrossing of a GMP. Damage may be purely economic or it may adversely impact on conservation assets. It is difficult to assess the exact level of damage that may be caused. However, the following basic assumptions may be useful for establishing monitoring priorities for a selection of GMPs that may become feral.

Signs of high damage potential

The likelihood of damage occurring increases

- with the degree to which the GMP tends to grow in the wild (high germination rate, long seed viability, no particular site requirements),
- with the frequency with which the GMP tends to not only grow temporarily but form self-perpetuating populations from spontaneously occurring plants,
- with the degree to which the plant in its GM-free variant is already showing signs of uncontrolled growth and spread (e.g. as a weed or invasive neophyte),
- if spontaneous populations are found adjacent to conventional crops of the same plant species, or
- if the local flora contains wild counterparts for hybridisation that are growing in the vicinity of spontaneous GMP populations.

Potential decision-making path

Based on these criteria, Table 2 presents a decision-making path for assigning to two priority levels those GMPs that are potentially capable of becoming feral:

No.	Criterion	Proceed to No.
1a	From experience, the tendency or otherwise to become feral can be quite accurately judged because the plant species frequently occurs or is even cultivated in the survey area (in its GM-free form); therefore seeds or other propagules are present everywhere.	2
1b	From experience, it is difficult to judge the tendency or otherwise to become feral as the GM-free form of the plant species occurs rarely or not at all.	3
2a	The plant species can regularly or frequently be seen growing spontaneously (possibly only as a volunteer crop on arable land). The rate at which randomly dispersed propagules (seeds or others) establish is relatively high.	3
2b	Spontaneous growth is however only rarely observed. For some reason, the rate of spontaneous establishment from randomly dispersed propagules is very low.	**Priority 2**
3a	Spontaneously occurring plants continue to be present at the same location for a period of at least several years; local populations may be self-perpetuating and persistent (established population).	4
3b	Spontaneously occurring plants soon die and are a transient presence. They only occur temporarily and certainly do not represent a persistent, self-perpetuating population.	5
4a	The local wild flora contains wild-growing counterparts for hybridisation. Outcrossing is generally possible.	**Priority 1**
4b	The progressive expansion of the spontaneous population as a result of vegetative or generative propagation (symptoms of establishment, dispersal or even invasive behaviour) is a plausible scenario.	**Priority 1**
4c	Neither 4a nor 4b apply, e.g. due to low competitiveness, susceptibility to local climatic factors or because the species does not produce seeds capable of germination or other viable propagules and is not capable of vegetative propagation.	**Priority 2**
5a	Spontaneous growth occurs only under very special conditions, rendering it a rare event by default, e.g. where the species requires site conditions that are rarely met (extraordinarily high soil moisture or deep burial in the soil).	**Priority 2**
5b	There are no significant limitations to spontaneous growth; or where specific site conditions are required these can generally be found in the vicinity of agricultural crops or nature reserves.	4

Table 2: Cascade of decisions to determine the urgency of a monitoring of undesirable populations of GMPs. Start with Criterion No. 1. By deciding on one of the options per question/criterion the user is taken to the next question and eventually to either Priority 1 or Priority 2.

3.4 Release pathways and locations

Monitoring of undesirable populations of GMPs should focus on locations at which there is a high probability of GMPs actually occurring. Only then will monitoring be efficient (see Chapter 7). It is useful therefore to take a closer look at release pathways and the locations at which GMPs tend to be released into the environment (Pascher et al. 2017).

The first step is to determine which goods contain (seeds of) the GMPs in question ("Identifying the goods" section). The following step involves tracing the routes taken by these goods, and their final destination. Somewhere along this route the

GMPs are unintentionally released ("Exposure pathways" section). At the end of this process one will have obtained a good general indication of the locations in the landscape at which the GMPs enter the environment ("Release locations").

Identifying the goods

The decision-making path set out in Table 3 can be used to assess whether random imported goods may contain GMPs of relevance to the monitoring programme. The starting point of the decision-making path (Criteria 1 and 2) takes account of the different regulatory frameworks for GMO monitoring in different countries. In the EU Member States it is important to differentiate between approved and unapproved GMPs. Monitoring plans are only mandatory for approved GMPs, but then without exception. In other countries, for example in Switzerland, no such differentiation is made and all spontaneous populations of GMPs are monitored regardless of a potential approval.

No.	Criterion	Proceed to No.
1	Are the goods destined for importation into an EU Member State and have the goods been declared to contain or potentially contain GMPs approved for importation and processing? Yes:	**Monitoring is mandatory (EU Member States)**
	No. If at all, the goods contain GMPs that are not approved. Or the goods contain approved GMPs which have not been declared or are contained in the goods as a result of accidental contamination.	2
2	Did the goods originate in a country in which GMPs are cultivated that are approved for importation and processing? Yes:	5
	No, but: The differentiation between approved and unapproved GMPs is immaterial for planning the monitoring programme, and GMPs are under cultivation in the country of origin.	5
	No. No GMPs are under cultivation in the country of origin, or only approved GMPs are relevant for planning the monitoring programme.	3
3	Are the goods in question processed products or mixed products consisting of multiple components, with individual components potentially containing GMPs capable of spontaneous growth (e.g. animal feeds, seed mixes, fireworks) and potentially originating in other unknown countries? Yes:	4
	No:	--
4	Will the possible mixtures of plant material continue to be capable of germinating and/or growing following pre-processing? Yes:	6
	No: The goods were processed in such a way (e.g. heat treatment, grinding, pressing) that any plant material present will no longer be capable of germinating and/or growing.	--
5	In the country of origin, are there GMPs under cultivation that are capable of spontaneous growth (within the meaning of Section 3.2), or are there any known cases of spontaneous populations of such GMPs in the country of origin? Yes:	6

	No: Contamination with GMPs capable of becoming feral is unlikely.	--
6	Have there already been any known cases of GMPs* capable of spontaneous growth having contaminated the goods in question? *Contaminations involving the plant's GM-free form or closely related plant species are similarly relevant in this context. → Experience shows that contamination is possible. Yes:	**Contamination is plausible**, Proceed to 9
	No:	7
7	Are the imported goods agricultural products in the broader sense (including their processed form) and, if yes, are they cultivated on the same land in the country of origin as the GMPs capable of spontaneous growth? → Contamination during harvest due to volunteers or outcrossing. Yes:	**Contamination is plausible**, Proceed to 9
	No:	8
8	Are the imported goods stored in the same locations or even in the same containers which had previously been used to store GMPs capable of spontaneous growth? → Contamination occurs during the transfer, packaging or loading of goods. Yes:	**Contamination is plausible**, Proceed to 9
	No: Contamination with GMPs capable of spontaneous growth is unlikely.	--
9	In line with their intended use, the goods are disseminated in the environment (seed, feedstuffs, straw, decorative material, fireworks, any others). Yes:	**Goods relevant for monitoring**
	No:	10
10	Are the goods transported in open bulk containers or at least in containers that are not hermetically sealed? Yes:	**Goods relevant for monitoring**
	No, the goods are transported in hermetically sealed containers. → Spillage of GMPs during transportation is hardly possible.	11
11	The containers used to transport the goods are emptied, unloaded or transferred to other containers in unprotected locations, i.e. locations that are not shielded from the environment (e.g. open switchyards). Yes:	**Goods relevant for monitoring**
	No. Following the border crossing, containers used to transport the goods are opened only at the processing location in a covered facility that is shielded from the environment. → Losses of GMPs in handling are unlikely or low.	12
12	Residues from the cleaning of goods are destined for further use and may enter the environment (e.g. in the form of animal feed). Yes:	**Goods relevant for monitoring**
	No. Residues from the cleaning and storage of goods (e.g. residues from sieving or filtering) are rendered harmless and disposed of securely.	--

Table 3: Control questions designed to assess whether an imported product is or is not relevant to the monitoring of spontaneous undesirable populations of GMPs. Start with question No. 1. A "Yes" or "No" either leads to the next question or concludes the assessment with the result as stated. "Goods relevant for monitoring" means that GMPs capable of spontaneous growth may possibly be released into the environment in the course of handling the goods. "--" = the goods or GMPs potentially contained therein are not relevant for GMO monitoring.

Following the assessment set out in the previous section, we will now look only at goods which may contain GMPs, and only those GMPs that justify a monitoring of their spontaneous populations, because:

- the GMP in question is approved for importation and therefore is subject to mandatory monitoring in any case (applicable in EU Member States), or because
- there is an elevated probability of the GMPs contained in these goods to grow spontaneously or even become feral and, over time, cause environmental damage.

Exposure pathways

There are a number of different possible causes for GMPs actually entering the environment following the goods' importation:

a) Unintended loss during transportation

- along the transport routes,
- in switchyards,
- in storage or processing facilities.

b) Targeted dissemination of goods, generally in line with their intended use

- agricultural cultivation from seed (arable crops, meadows, lawns),
- dissemination in the form of feed in livestock housing, feeders, aviaries etc.,
- use as bedding in animal enclosures,
- use as decorative material, e.g. cut flowers, floristic arrangements,
- use of fireworks (may contain seeds of oilseed rape).

The causes of the release of GMPs into the environment are important for the relative urgency of monitoring undesirable populations of GMPs. If goods are disseminated in the environment in a targeted manner (case b) even small product quantities may result in the release of GMPs into the environment. A single 300g package of a bird seed mix used to feed birds in the park is equivalent to a 15 ton container spilling 0.02 per mil of its cargo in transit along a railway line. Therefore, goods that are disseminated into the environment in a targeted manner are of particular importance to the monitoring of undesirable populations of GMPs.

If a product contains GMPs, this does not necessarily imply that the GMPs will actually enter the environment. Food items in particular are often so well packaged that no materials can enter or exit the transported goods. As long as the containers are not opened from the point of origin to the processing location, the risk of transport losses is very low even if the cargo contains GMPs. Therefore, the control questions 8 to 11 in Table 3 assess whether or not the means of transport or packaging could result in the release of transgenes. For them to enter the environment, the GMPs would need to be spilled from a package or container. Critical factors in this respect are:

- open or not tightly closed containers,
- transfers into other containers,
- containers or vehicles onto which GMPs had spilled becoming dispersal vectors,
- unpacking and bagging of goods at the point of destination,
- cleaning of containers that had contained GMPs,
- disposal of residues subsequent to the filtering or cleaning of goods.

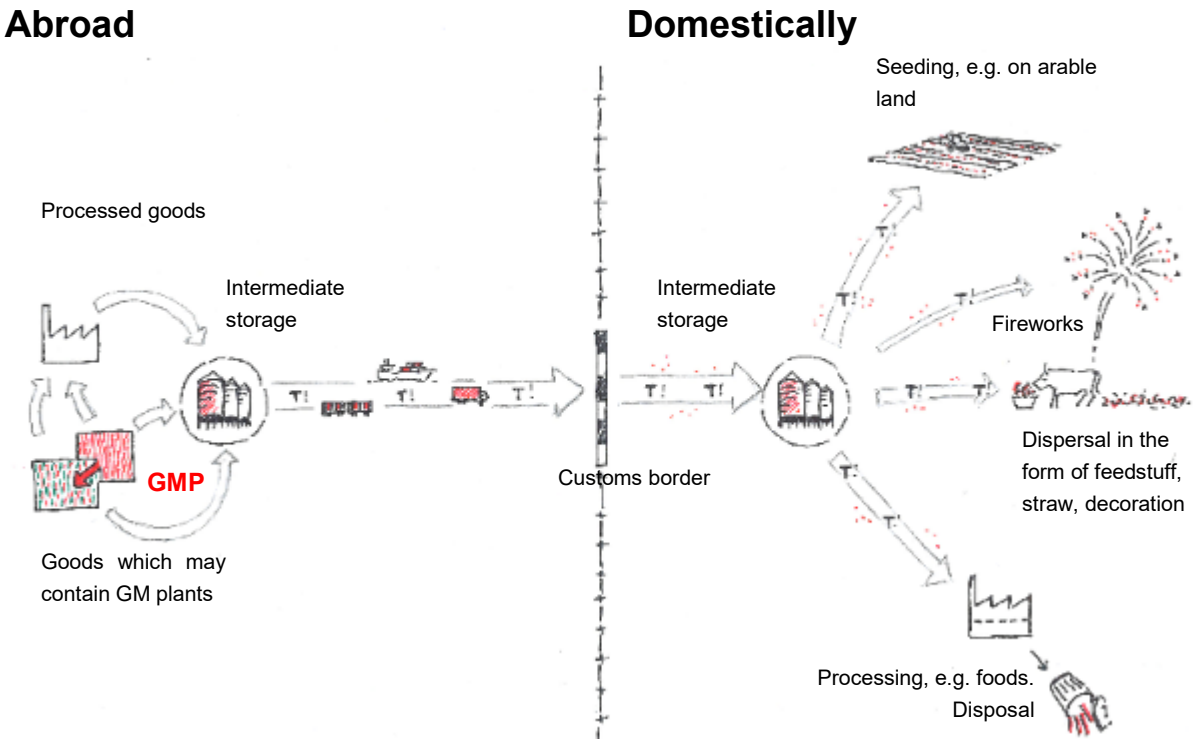


Fig. 4: Illustration of the potential pathways of distribution of GMPs. It should also be noted that the transport routes (T!) and intermediate stations are important with regard to all exposure pathways and all types of goods (seed, feedstuffs, foodstuffs). The presence of transgenic plant material capable of spontaneous growth is denoted in red.

Release locations

The exposure pathways described above lead directly to the release locations (Fig. 4). These locations are basically determined by the exposure pathways (see above). Generally these are:

- the transport routes per se (e.g. road network, rail network),
- intermediate stations along the transport routes (harbours, railway stations, storage facilities),
- premises of processing operations,
- locations of targeted release (in the case of seed, feedstuffs, decorative material, fireworks; see Section 4.3).

In planning a monitoring programme for undesirable populations of GMPs, the challenge is to prepare a very specific list or map of survey areas. They need to depict those locations in the landscape at which there is a highly elevated probability of GMPs growing spontaneously. These are the locations on which the monitoring programme will focus. Cost-efficient monitoring can only be conducted if these locations are known. In many cases it is only possible to narrow down the release locations when initial results of empirical studies become available. With ongoing analysis of monitoring results, monitoring efficiency can gradually be increased (see Chapter 7).

So how can an initial list of specific potential release locations be drawn up at the outset of monitoring? A number of aids can be used to this end:

- commercial register excerpts,
- membership lists of trade associations,
- border control data or databases of customs authorities,
- lists of cargo ports on the seaboard and along navigable rivers,
- searches in telephone directories and Yellow Pages,
- importers' client bases,
- searches for company websites on the Internet.

4 Examples of monitoring modules

Once the clarifications described in Chapter 3 have been completed, the GMPs and goods flows that should be prioritised for monitoring have been identified. Now it is time to specifically plan the detailed design of the search for undesirable populations of GMPs.

The search for spontaneously growing GMPs must be planned specifically for each target GMP, i.e. it must be adapted to the situation in the survey area. However, certain circumstances will be encountered repeatedly in different situations, giving rise to similar monitoring approaches. This chapter outlines examples of such "building blocks" of GM monitoring programmes. These are mostly targeted at certain types of release locations. While the monitoring approaches presented here originated in Switzerland and Germany, they may well be readily adaptable to situations found in other European countries.

Basic principles for all modules

GMP monitoring should be clearly structured both in space and time, i.e. survey areas should be named and delimited unambiguously. This make it clear where searches for GMPs have or have not been conducted and to which parts of a country or a landscape the monitoring results apply. Various other methodological characteristics of a monitoring programme must also always be defined. Accordingly, the individual modules presented below are structured uniformly. This better reveals their distinguishing properties. The following structure can be used as a blueprint for planning a monitoring programme and helps to purposely define its essential characteristics.

- Definition of the survey area (characteristics, challenges);
- Structuring of the survey area (possibly definition of stages to be implemented over time based on defined priorities, e.g. individual transport axes prioritised by location and traffic volume);
- Objects for sampling ("monitoring units", e.g. road segments, arable plots, railway stations);
- Notes on sampling (special characteristics);
- Short summary of investigations already conducted and their results.

4.1 Module: Railway network

This module serves to detect spillages of goods containing GMPs along railway tracks, i.e. resultant spontaneous populations of GMPs. This module has been in use in Switzerland since 2014 and is targeted at GM oilseed rape. The following principal framework conditions apply to the railway network, at least in Switzerland:

- Herbicides are applied regularly to the railway track as part of its management. The vegetation cover is generally sparse. Herbicide tolerant plants are evident.
- Therefore, GM oilseed rape populations tend to be young and relatively homogenous in terms of their size and developmental stages.
- In Switzerland, a single railway track is often used for railway traffic in both directions. In such cases there is little difference between the two sides of the track in terms of the frequency and density of spontaneous populations of oilseed rape.

Aim

1. Efficient detection of spontaneous GMP populations along the railway network.
2. Estimate of the frequency of undesirable populations (general distribution) of GM rapeseed lines along the railway network.

Definition of the survey area

Spontaneous populations of GMPs could theoretically occur along the entire railway network. The Swiss railway network has a total length of 5630 km. GMP monitoring is limited to sections of the network, totalling 1340 km. Surveys are conducted along the Swiss federal railways' (SFR) interoperable railway network. The interoperable tracks comprise those sections of the SFR network that meet the minimum technical requirements required for barrier-free passage of trains originating in other countries' networks. This part of the railway network contains the most important import and transit axes, especially the North-South and the West-East axes that run through Switzerland. The assumption is that on account of the flow of goods through these axes, this part of the railway network will be more strongly affected by spontaneous populations of GM oilseed rape. Therefore the monitoring programme does not include many of the regional tracks and narrow-gauge tracks.

Structuring of the survey area

The monitoring of the railway network is subdivided into two components:
Part 1 - highest priority: The selection of network sections included is risk-based. The focus is on aim No. 1 (detection).
Part 2 - standard programme, not risk-based: Random sampling of network sections over the entire survey area of 1340 km of railway track length. This part focuses on aim No. 2 (estimate of frequency).

This division is also useful because there are different processes involved in transport losses of GM oilseed rape. Firstly, seeds scattered on the cargo train when containers are loaded get blown away or shaken off as the train is moving.

Presumably the number of seeds spilled strongly and rapidly diminishes with increasing distance from the source of import (see Yoshimura et al. 2006 for road transport). These transport losses are recorded by Part 1 of the monitoring. Secondly, during rail transit and primarily due to vibration seeds enter the environment as they are lost from containers that are not fully sealed. This can be an ongoing process, resulting in seeds being dispersed along the entire transport route. Such losses are recorded by Part 2 of the monitoring.

Objects for sampling

Prior clarifications had shown that, firstly, the transshipment of loose bulk cargo at the Rhine Port in Basel is a significant source of the undesirable dispersal of seeds. Secondly, it was found that durum wheat from Canada is the most significant cargo in quantitative terms and that it is indeed contaminated with GM oilseed rape seeds. The first part of the survey concept, in particular, is based on these findings.

Part 1 (risk-based): A small number of railway tracks must be considered for the monitoring programme. These tracks should be surveyed repeatedly at intervals of no more than a few years. These include the access tracks to the major transshipment sites for oilseed rape in Switzerland: a) the Rhine Port in Basel and b) the only two existing oilseed mills in Switzerland that process rapeseed. In addition, the focus is on c) the transport route from the Rhine Port in Basel to the buyers of the durum wheat, i.e. two grain mills in the Aarau – Zürich region. From Basel onwards, the durum wheat is transported exclusively by rail.

Part 2 (random): The sampling strategy involves the annual investigation and sampling of 30 randomly chosen sections of the tracks of 1 km length each. Approximately 2% of the survey area will therefore be covered per year which should be sufficient to provide robust data (see Chapter 7). The sections are located at regular distances of approximately 45 km from each other, dispersed over the entire survey area of 1340 km of track length. The first location is chosen at random and constitutes the starting point for all other sections of the track network to be surveyed.

The annual minimum length of 30 km of track to be monitored per year for Part 2 of the monitoring was deduced from initial estimates of the frequency of oilseed rape and GM oilseed rape. This estimate is based on the results of the first, risk-based stage of the monitoring programme but was modified to account for the situation along the randomly selected railway tracks. An average density of 10 oilseed rape plants per kilometre of railway track is expected, while 1% of the total population is anticipated to be genetically modified. Error probability, i.e. the probability that GM oilseed rape is overlooked in the course of sampling or that it is not detected during analysis, was established at $p = 0.05$.

Notes on sampling

So far, a sample has been taken from each of the oilseed rape plants found in the sampled areas. Were the density of oilseed rape plants at a location to exceed 30 individual plants per square metre, only a random sample of individual plants would be taken. Standardised procedures are used for plant sampling and

analysis of samples in the laboratory. Further information on these standards is given in Chapter 6.

Summary of investigations conducted in Switzerland to date
Part 1 (risk-based):
Over a period of two years, nine sections of railway track along the access routes to the Rhine Port in Basel and the oilseed mills were tested for the presence of oilseed rape. On a total of 40 km of track that has been checked, 655 oilseed rape plants were found, of which 134 (20%) contained a transgene conveying herbicide tolerance. These GM plants were found in two separate locations containing 21 and 113 GM oilseed rape plants respectively.

Along the railway lines between the Kleinhüningen Port and the oilseed mills, oilseed rape samples were taken at 18 specifically selected sites. Sixteen of these sites were sections where the tracks curved or which had a high density of railway switches (railway stations). There is a heightened risk of spillage of loose seed material at such locations. The individual sections are between 400 and 1000 m long and have a combined length of 9150 m. In addition, two selected sites (major junctions) were sampled at the shunting yards at Muttenz and Limmattal respectively. This is where the cargo carriages containing the grain are sorted into complete cargo trains. Spontaneously growing oilseed rape plants were found in all of the 18 sampled track sections, with individual sections containing between 7 and 313 individual plants. However, none of the total of 1314 sampled plants were found to contain a transgene.

Part 2 (random):
In the three years from 2014 to 2016, three times 30 km of track (a total of 90 km) were surveyed in accordance with the survey strategy. A total of 1922 oilseed rape plants were found and sampled. The oilseed rape plants do not occur evenly scattered but are clearly clustered. The kilometre of track hosting the oilseed rape population with the highest density of plants included 205 individual plants. On 37 km or 41% of the 90 km of track surveyed, no oilseed rape plants were found at all. Of the total of 1922 plants, laboratory analysis confirmed 9 plants to be herbicide tolerant GM oilseed rape (0.5%) that is resistant to Roundup. These 9 plants occurred in two separate locations hosting 7 and 2 GM oilseed rape plants respectively.

4.2 Module: Road network

A GM monitoring programme for the road network primarily records transport losses during road transport. Similar to the railway network module it should be used where goods potentially containing GMPs are transported in containers that are not fully sealed or where they are transported as loose bulk goods on lorries or where vehicles could become vectors for the distribution of loose material left behind on load spaces. A monitoring module for a road network has not yet been implemented anywhere. However, preliminary works and considerations have been undertaken as to the design of such a module. These are summarised below.

Special conditions apply to the recording of GMPs along road networks and these may differ between different survey areas. The conditions in Switzerland are as follows:

- Management of roadside verges and embankments normally consists of mowing. Herbicide applications are prohibited. Therefore, roadside vegetation tends to be rather dense, persistent and complex.
- For this reason it is entirely feasible for multiannual, persistent populations of spontaneously growing GMPs to occur.
- Moreover, GM oilseed rape populations alongside roads are exceedingly heterogeneous in terms of plant sizes and developmental stages.

Aim

1. Efficient detection of spontaneous GMP populations along the road network.
2. Estimate of the total population of GMPs along the road network.

Definition of the survey area

Similar to the railway network, the survey area consists of linear sections connecting nodal points (loading facilities, crossroads, access roads, roundabouts, petrol stations, service areas etc.). Transport losses of GMPs are likely to primarily occur in areas that are 1. regularly frequented by a high number of lorries, and 2. frequented by lorries transporting goods which may contain GMPs. Using these two criteria, sections of the road network can be identified the total length of which is practicable for monitoring purposes.

The Swiss road network has a total length of more than 70,000 km and contains countless nodal points. Given their different functions and traffic volumes, the scope of a survey in Switzerland could be defined on the basis of the following road types:

- National roads: 1892 km; main corridors and transit axes of national and international importance.
- Main roads: 2262 km; cantonal roads, primarily for through-traffic and serving important regional and touristic connectivity functions[8].
- Special types of backroads which may chiefly host GMP populations which have become feral. Primarily these are the first kilometres of access roads to and from cargo loading and handling facilities (also see further below).

The first two types of roads carry a major share of the heavy goods vehicle transport in Switzerland. Almost regardless of the GMPs chosen for monitoring, these roads are likely to be significant for GM monitoring along the road network, given their generally high traffic volumes. In contrast, the special types of backroads are chosen for characteristics other than high traffic volume. Their individual selection is dependent on the choice of GMP to be monitored (e.g. entrance points, cargo handling facilities, purchasers' company premises).

Structuring of the survey area

The basic premise is that the distance to the handling facility for goods containing GMPs is the most significant factor explaining the presence of spontaneously

[8] Pursuant to the 2007 'Regulation on the application of the earmarked Mineral Oil Tax (MinOA) in road transport' (*SR 725.116.21 Verordnung über die Verwendung der zweckgebundenen Mineralölsteuer im Strassenverkehr vom 7. November 2007*).

occurring GMPs (Bailleul et al. 2012). While other factors are not yet known, these are to be established in the course of the monitoring programme and should increase its efficiency. In analogy to the railway network module, it is useful to subdivide the monitoring efforts into two layers at the outset. For both layers, sections of the network will be randomly selected and surveyed:
Layer 1 – targeted search at main suspect locations: The selection of roads comprises a relatively small number of sections at which there is a maximum probability of the occurrence of GMPs. The primary focus of this layer is to meet aim No. 1.
Layer 2 – supplementary search in the remaining survey area: This work concerns the entire remaining scope of the survey, which generally would be the greater part. The primary focus of this layer is to meet aim No. 2.

The Layer 2 results mainly serve to identify the factors determining the occurrence of GMPs. The more successful this work, the more the subdivision into the original two layers will be rendered obsolete, as sampling along roadsides can increasingly be targeted at sections where there is a high probability of the occurrence of GMPs. Nonetheless, the total population of GMPs can still be estimated, provided the basic principles of adaptive design are adhered to (see Chapter 7).

Objects for sampling
In accordance with the results of pilot surveys on populations of spontaneously growing oilseed rape plants in Switzerland, suitable road section units (objects) for sampling could be defined as follows:

Part 1 (risk-based): Targeted selection of feeder roads to handling or processing facilities for goods containing GMPs. Pursuant to the results obtained by Yoshimura et al. (2006) for oilseed rape, a minimum length of 2 km and preferably 5 to 10 km of roads from the handling facility should be monitored.

Part 2 (random): Annual survey and sampling of a random selection of road sections of 100 m length each. The length of the sections can be adapted to the conditions prevailing in the survey area (see the notes further below) but should be uniform.

The length of the sections for sampling should
- contain a manageable number of individual plants for sampling,
- be sufficiently small to allow for the unambiguous spatial assignment of important site characteristics (e.g. curves, site entrances, service areas) and to ensure that these are reflected in the data analysis,
- be sufficiently large to justify the time needed to visit the section (labour efficiency).

Data on the distribution of spontaneously growing oilseed rape alongside roads in Switzerland show that segments of 100 m length meet these criteria well (see results further below). Where the density of individuals is low, it is efficient to survey a number of adjacent 100 m segments, e.g. five segments each in groups of 500 m. This would reduce travel times between sampling sites.

Which area along the road verge should be surveyed? The bulk of spilled seeds drop to the ground in the immediate vicinity of the road (Bailleul et al. 2012). Moreover, persistent populations are primarily to be expected in the areas of the verges that are largely free of vegetation. Therefore the area to be considered for sampling along the road should have a width of approximately 1 to 3 m. Its positioning is shown in the diagram by Nishizawa et al. (2009) ("monitoring zone" in Figure 5):

- Road verge between the asphalt pavement and the curbstone including the drain ("side strip")
- Area around the crash barrier or edge of the road
- Uncultivated areas directly adjacent to the road/crash barrier.

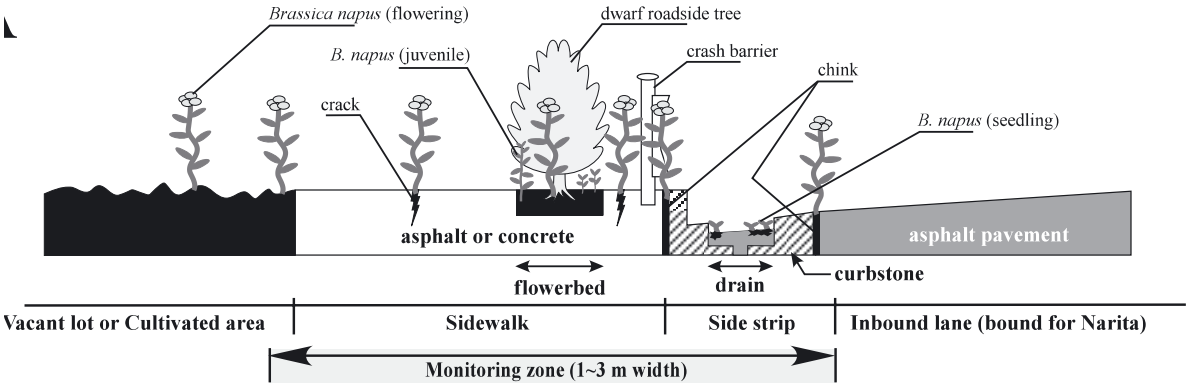


Fig. 5: Diagram showing the different kinds of sites along roadsides. After Nishizawa et al. (2009).

What is the total road length that should be monitored annually in order to yield robust data? The answer to this question is determined by a number of different factors, the most important of which are the frequency and distribution of GMPs in the survey area, metrics that are unknown at the outset of a monitoring programme. For a rough estimate, we assume that GMPs are very rare, i.e. that they occur on less than 1% of the road sections, and that an adaptive sampling design is used for sampling and data analysis. An annual total effort of approximately 2% of all available road sections should be sufficient. Details on adaptive sampling and on how this "rule of thumb" has been derived are given in Chapter 7. In the initial stage of the monitoring programme, this total effort should be split roughly evenly between the two components of monitoring (1. risk-based selection layer, 2. random sample layer). Once the monitoring has, over the years, yielded more detailed knowledge on the distribution and frequency of GMPs, the sample can be composed differently.

Notes on sampling

Caution: There is always a possibility that the transport routes for certain goods are unidirectional. This means that the distribution and density of spontaneous plant populations differ by the direction of travel and thus between the two roadsides. This is true at least for Switzerland.

The approach to sampling potential GMPs detected in the field is the same as for the railway network module. The same standards apply (see Chapter 6).

Summary of investigations conducted in Switzerland to date

As part of preliminary investigations for the road network module, a total of 125.8 km of road verge in Switzerland were surveyed for spontaneous populations of oilseed rape. These included 14 different road sections, some of which were rather long: 98 km along national roads (motorway) and 27.8 km along main roads, with the two sides of the road in a given section being counted separately. The study was undertaken in 2014 and 2015. Most of the sections were visited both years. Significant findings include the following:

- Spontaneously occurring oilseed rape was detected in all of the 14 road sections surveyed.
- Oilseed rape plants were present in all possible stages of plant development, i.e. from seedling to rosette stage to flowering and fruiting plants.
- None of the leaf samples collected indicated the presence of GMPs. Tests were conducted for three different herbicide tolerance events (T45, MS8/RF3, GT-73).
- Oilseed rape plants occurred at median densities of 3.9 individuals per hundred metre along motorways and 2.3 individuals along main roads. This median value varied between 2.3 and 4.8 plants per hundred metres depending on the section sampled and the sampling year.
- The median density of oilseed rape populations (groups of plants) was 1.5 and 1.1 populations per 100 m along motorways and main roads respectively. This median value varied between 0.9 and 1.8 plants per hundred metres depending on the section sampled and the sampling year.
- The distribution of oilseed rape over the entire route was highly uneven. At least the observed number of oilseed rape plants per 10 m transect diverged from the expected Poisson or normal distribution. More than 85% of transects along the motorways contained no oilseed rape, while individual transects contained up to 41 individual plants.
- While certain regions and motorway sections were shown to have a generally higher tendency to host oilseed rape populations, these findings can be relatively well explained by their distance to facilities that process oilseed rape, by domestic cultivation of oilseed rape, and by collection points for harvested oilseed rape. However, the number of oilseed rape plants in the same section varied strongly between years.
- Road verge surveys in the Canton of Ticino – where oilseed rape is not cultivated – indicate that transport losses from lorries are relatively rare.

4.3 Module: Seed, food and feed

Hygiene and purity are key concerns in the modern production of seed, food and feed. Therefore, quality control systems monitoring contents of undesirable components and substances are in use in many countries. These systems generally also include checks for GM content. Given these circumstances, a new monitoring module for GM plant contents of food, seed and feed that is separate from existing controls would not make sense. Existing systems should be improved and gaps filled instead. This chapter sets out key aspects to be taken into consideration.

Aim
Optimisation of existing control systems for seed, food or feed in order to ensure that items containing GMPs capable of spontaneous growth can be more reliably identified. The controls allow for goods containing GMPs to be withdrawn from the market before the GMPs can enter the environment.

Definition of the survey area
The survey area is not spatially delimited. The "survey area" consists of the quantity of goods present in a given country with the exception of foodstuffs transported in small units as these have a high degree of purity, are not transported in bulk and are generally contained in hermetically sealed packaging.

Structuring of the survey area
Distinctions may be made in terms of the goods' origins:
1. Imported goods
2. Domestically produced goods

If there is no domestic cultivation of GMPs, controls can be limited to imported goods. However, if there is domestic cultivation of GMPs, and especially if GMP seed is produced, domestically produced goods must also be monitored. The selection of goods to be monitored is dependent on the considerations set out in Chapter 3.3 and 3.4 or on statutory provisions.

Gaps in the control system

Where the search for GMPs has already been integrated into existing control systems for seed, food and feed, the focus tends to be on agricultural crops of which a transgenic cultivar is in existence. However, apart from this "classic" situation, there are at least two additional situations giving rise to the potential presence of GMPs. So far there has been a high probability of both of these situations being overlooked. Gaps like these must be identified and filled by introducing relevant controls.

For seed, for example, existing controls primarily target important agronomic traits such as varietal purity and low weed seed content. Until recently, controls of grass seed in Switzerland were significantly less strict than controls of arable crop seed. In the case of feedstuffs for captive animals, e.g. birdseed, there were no controls whatsoever. The aim of preventing the spontaneous release of certain species, which may be present only in the form of minor admixtures, called for an adjusted approach.

1. Impurities in agricultural products
Known cases of GMP admixtures to agricultural products declared as GM-free were cases of intra-species contamination, e.g. GM linseed from Canada in linseed shipments to Europe (see e.g. Price & Cotter 2014[9]). While coexistence of GM and non-GM alfalfa cultivars for alfalfa hay production in the USA is

[9] http://www.gmcontaminationregister.org/

considered to be possible under certain circumstances, low-level contamination cannot be ruled out entirely (Putnam et al. 2016). Inter-species contamination of agricultural products has as yet rarely been detected; however, checks for this type of contamination are probably conducted rarely, if ever. Nonetheless, there are examples of GMP impurities in agricultural products:

- GM oilseed rape was found in France in a shipment of mustard seed (*Brassica juncea*) from Canada. The level of GM oilseed rape present in the mustard seed was only 0.003% (Demeke et al. 2006).
- GM oilseed rape was found in Switzerland as an impurity in durum wheat shipments from Canada (Schulze et al. 2015). Seed spillage resulted in spontaneous occurrences of GM oilseed rape. The durum wheat has also been found to be contaminated with other plant species, among them alfalfa (non-GM, pers. comm. KLBS, C. Bagutti).

The general lack of checks for GMP impurities is a major shortfall of existing control systems. The small number of published studies on the issue indicates that impurities are a regular occurrence, at least when it comes to feedstuffs (van Denderen et al. 2010). Seed is also checked for impurities but not specifically for the presence of genetically modified seeds.

In light of the thousands of tonnes of food and feedstuffs, the issue of impurities should not be underestimated. Impurities are a significant factor in spontaneous releases, especially where a) large volumes of goods are moved, and b) where products enter the environment in a targeted way, i.e. in the case of hay, straw, pet food, livestock feed, or seed.

2. Components of unknown origin

There are gaps in the control systems in cases where the origin of goods is unknown. Globalised trade and low transport costs have resulted in a situation where the components of a processed product may have originated in different parts of the world. Unless such products are food or feedstuffs it is likely that there are few if any controls. In such cases the origin often does not need to be declared which makes it very difficult to recognise the product as potentially containing GMPs.

One example of goods of this nature are seed mixes used to feed birds. These are purchased by private individuals to feed their pet birds at home or wild birds outdoors. Birdseed mixes contain seeds of a range of plant species such as hemp, flax, canary grass, milo and proso millet as well as seeds of oilseed rape and field mustard. These mixes are imported from abroad and it is almost impossible to establish the origin of their individual components. Investigations in Switzerland have shown that such birdseed mixes regularly contain GM oilseed rape and may be a source of spontaneous populations of GM oilseed rape in the wild (Innovabridge 2016, 2017). The study investigated a total of 40 packages of birdseed mixes which had been purchased from retail outlets. Important findings of this study are as follows:

- 37 of the packages contained seeds of the *Brassica* genus. GM Brassica seeds were detected in 23 ouf those 37 packages (62%). Several packages contained transgenes by different, competing companies.
- 8 of the packages containing GM seed were selected for a more detailed investigation, estimating the proportion of GM oilseed rape seeds as well as

their germination rate. Germination rates were high, varying from 61 to 96% between packages. The proportion of viable GM oilseed rape seed to total oilseed rape seed varied between 0.4 and 20.4% between packages.

- A single package of one kilogram of birdseed mix may therefore contain more than 7000 viable GM oilseed rape seeds. A rough estimate of the number of viable seeds of oilseed rape released into the environment every year in Switzerland by people feeding birds is in the order of 37 million. If these seeds are not consumed by birds, they may grow into oilseed rape plants in the wild that are capable of reproduction.

A second example of goods not commonly expected to contain GMPs are fireworks. The majority of commercially available fireworks are produced in non-EU countries. Fireworks use rapeseed coated with metallic salts in order to achieve the "starburst effect". In trials, up to 30% of the rapeseed were found to be viable seed. Given that a small percentage of the seeds do not incinerate when the fireworks explode, it is possible that up to 10 million viable seeds are dispersed in Germany every year[10]. No research has been conducted as yet to ascertain whether this rapeseed is genetically modified or not. From a legal point of view such controls are not currently mandatory – a gap in the control system. There is no evidence as yet of any spontaneous populations of oilseed rape originating from fireworks. Nonetheless, fireworks are a surprising example of unexpected pathways through which – in conjunction with gaps in the control system – viable GMPs may be imported and dispersed. However, according to information provided by pyrotechnics manufacturers plant seeds are only rarely used nowadays in the production of fireworks. In 2014, the authorities in Switzerland conducted a one-off inspection of approximately 70 fireworks; no seeds were detected in these fireworks (mentioned in KLBS 2015).

Routine controls: The example of seed in Switzerland

In Switzerland, a general import permit[11] is required for the importation of seed of various types of crops, including seeds of wheat, rice, barley, oats, triticale, field mustard, sugar beet, fodder beet, tomatoes, chicory, and cotton. Prior to importation, each individual shipment of seed of these crops must be notified in writing to the Federal Office for Agriculture (FOAG) for control purposes. A random sample of these shipments is subjected to routine quality controls, i.e. tests for purity, viability, impurities, and seed heterogeneity.

Switzerland recognised at an early stage that genetically modified plants could be introduced by means of contaminated seed and issued appropriate regulations in response. Since 2000, seed importers in Switzerland must have a quality assurance system in place designed to prevent contamination with genetically modified organisms. In addition, the FOAG has been checking seed imported into Switzerland for traces of GMPs. The seed shipments are tested for the presence of various traits contained in a range of different GMPs. Contamination with foreign GMPs would be detected in this manner. In addition to a standard screening for widespread traits, targeted searches are conducted for specific traits that are more

[10] www.taz.de/!5129530/ The article cites a study by Broder Breckling of the Center for Environmental Research and Sustainable Technology of the University of Bremen.

[11] www.blw.admin.ch/blw/de/home/markt/einfuhr-von-agrarprodukten/saatgetreide-und-saemereien.html

likely to occur in a given seed shipment. The latter searches are conducted on a case-by-case basis, tailored to the shipment in question.

The following selection criteria are used to decide which seed shipments are to be sampled:

- Seed originating in countries where there is widespread cultivation of GM plants are always checked;
- Seed originating in countries from which other seed shipments have already been found to contain GMPs are checked more frequently;
- Shipments from countries where there is no cultivation of GMPs and/or from countries in the shipments of which GMPs have never been detected are subject to random checks.

However, these routine controls are only conducted for seed of the crops listed above, i.e. those that require a general import permit. Until recently seed of all other crops had not been checked for the presence of GMPs. This gap became evident when an increasing number of genetically modified fodder and pasture plants were developed and approved in the USA. The first trial inspections of alfalfa seed were conducted in Switzerland in 2017.

Summary of investigations conducted on seed in Switzerland to date

Official inspections in Switzerland:
Imports of seed into Switzerland covered by general import permits are primarily maize imports. In 2016, the 458 shipments of maize constituted 85% of all imported seed shipments. The inspections carried out over the past 15 years have only ever detected GMPs in maize seed. The proportion of positive detection results in the total number of imported shipments is small: 313 maize samples were analysed between 2001 and 2016, with 8 samples containing an impermissible level of GMPs (BLW 2017). The inspections include an initial screening process for a broad spectrum of common GM events. Thus any contamination with GMPs other than maize would have been detected. It should be emphasised that the 313 analysed samples had been taken from seed shipments which were presumed to have an increased likelihood of containing GMPs. Therefore, the results do not allow for conclusions as to the overall proportion of maize seed contaminated with GMPs.

The first trial inspections of seed outside of the scope of general import permits were conducted on alfalfa seed from the USA and Canada in 2017. None of the samples tested positive. The efforts undertaken in the USA with a view to keeping separate the seed of conventional and GM cultivars would so far appear to be successful[12].

Voluntary inspections in Switzerland:
The Swiss association for seed trade and plant breeders' rights (Swiss-Seed) voluntarily developed a GM seed monitoring process (Swiss Seed 2016). It is applied to goods that are subject to special import conditions imposed by the Swiss Federal Office for Agriculture. At present these are maize, soya, turnip, oilseed rape, cotton, sugar beet and fodder beet. In line with the precautionary

[12] https://www.alfalfa.org/CSCoexistenceDocs.html

principle, the vast majority of seed importers apply the following to shipments of seed of the above species:

1. Each imported shipment is analysed in accordance with the analysis protocol issued by the governmental inspection body (Agroscope, Posieux).
2. Small samples may be analysed in the form of aggregate samples.
3. Samples are taken in accordance with ISTA standards by an appropriately trained and qualified person.
4. A copy of the analysis results is retained by the importer.
5. A repeat import of a lot already analysed does not need to be analysed again. The same applies to lots already inspected by the authorities.
6. Seed will only be placed on the market when the analysis results are available and no GMPs have been detected.
7. All results, including results relating to lots tested by FOAG, can be accessed by Swiss-Seed members.

4.4 Module: Processing and transshipment facilities

The module on processing facilities is concerned with spontaneous GMP populations originating at the arrival point as a result of the processing of goods containing GMOs. These are goods which either

- contain imports of authorised GMPs, or
- where the GMP content has not been properly declared, or
- which are contaminated with adventitious admixtures of GMPs.

The module on processing and transshipment facilities supplements the modules on railway and road networks which are targeted at spontaneous GMP populations alongside transport routes. Once the goods have reached their destination, they are unloaded and unpacked, possibly placed into intermediate storage, and subsequently filtered, cleaned, ground, pressed etc. It is conceivable, in principle, that in the course of these steps a proportion of the goods, including the GMPs they may contain, are released into the environment and that spontaneous, undesirable populations of GMPs develop as a result. Of particular interest for the monitoring programme are those companies that receive direct imports from countries where GMPs are cultivated on a commercial basis, or secondary imports through EU countries (Wedlich et al. 2016).

Aim

Efficient detection of undesirable GMP populations in the vicinity of processing facilities.

Definition of the survey area

Of interest for this module are all sites associated with companies and facilities at which goods potentially containing GMPs are handled. This imprecise description underscores the significance of the clarifications outlined in Chapter 3, the aim of which is to specifically identify goods that could potentially contain GMPs including their destination. Otherwise the number of processing facilities for monitoring would be unmanageably high. However, once the relevant goods have been identified, the survey “area” can be narrowed down. The focus should be on those companies and facilities that are highly likely to receive goods containing GMPs.

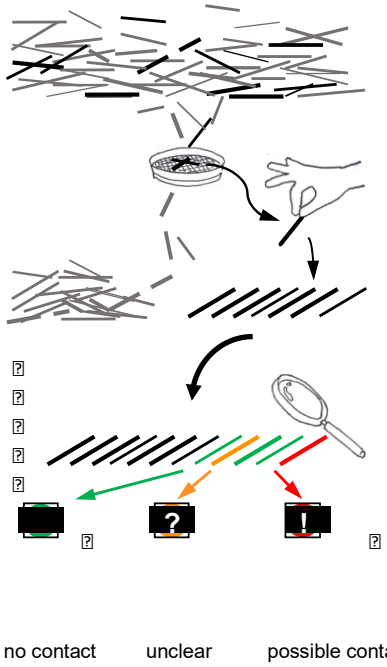
The development of a list of processing and transshipment facilities for the purposes of monitoring spontaneous GMP populations is an iterative process. Its starting point is an initial, fairly comprehensive list of locations at which goods potentially containing GMPs could be processed. A range of different information sources are available to this end which could also be used in combination. Not all of these sources will be available in all countries.

- membership lists of trade associations (e.g. feed merchants)
- border control data or databases of customs authorities,
- lists of cargo ports on the seaboard and along navigable rivers,
- a list of goods stations in the railway network,
- searches in telephone directories and Yellow Pages,
- a review of the client bases of importers and logistics companies,
- searches for company websites on the Internet,
- commercial register excerpts.

The criteria used to select processing facilities for inclusion into this initial list should be relatively broad. Any potential involvement with agricultural products in the widest sense should suffice. Once this initial list has been drawn up, the research process should proceed as follows (Fig. 6):

- Application of rough filter criteria, resulting in a manageable number of entries. Such criteria could include, for example, categories of goods or activities (e.g. oil mills in general, feed merchants). In a step-by-step process, ever more stringent selection criteria are applied until this goal has been reached.
- A check of each individual entry and decision with a view to whether it would indeed be useful for a monitoring programme. This decision is taken on the basis of a) the company name as listed in the information source and b) its operations, objectives and activities as described in the commercial register (e.g. oil mills processing oilseed rape).
- Identification of those entries, i.e. company branches or sites, that actually handle goods potentially containing GMPs (e.g. oilseed rape mills processing imported goods from Canada). Each individual remaining entry is once again assessed to this end. The necessary information must be obtained either directly from the companies or indirectly using the sources listed above. The result is a pragmatic classification of the entries into three categories:
  a) company does not handle relevant goods, i.e. no contact with GMPs;
  b) company handles relevant goods at least occasionally, i.e. potential contact;
  c) company potentially handles relevant goods, but this has not been verified.

Category c) and possibly Category b) constitute the initial survey area for the monitoring programme.



All addresses / entries:
- Customs database
- Commercial register
- Telephone directory etc.

Automated filter (e.g. keywords)

Limited number, i.e. less than appr. 1000 entries

"Manual" checking and shortlisting

Only entries of relevant sectors remain

Targeted research on each entry

Only entries with conceivable connection to goods containing GMPs

Allocation to 3 categories

Fig. 6: Approach to the selection of processing facilities to be included in GMO monitoring. Ultimately, the only addresses relevant to the monitoring programme are those at which there may be contact with goods containing GMPs (red and yellow "traffic light").

Structuring of the survey area

Even when using a restrictive definition of the survey area, the number of processing facilities of interest can still be in the hundreds. It may therefore be expedient to prioritise within the selection. If adaptive sampling design is used, (see Chapter 7) this is even a requirement. This may allow for a reduction of the survey area in the course of monitoring. Suitable criteria for deciding on priorities might include:

- the type and condition of the goods for processing which may contain GMPs (e.g. exclusively unprocessed soyabeans),
- the mode of transport and the type of container used for transport in which the goods arrive (e.g. goods exclusively transported in open bulk containers),
- the goods' precise intended use (e.g. animal feed),
- a threshold value with respect to the total processed volume of imported goods potentially containing GMPs,
- a threshold value with respect to the size of the companies' premises.

Objects for sampling

In general, every processing facility constitutes a distinct object for sampling purposes. In the case of very large or complex company premises it might be possible to define several sub-units (e.g. storage unit and production site) or to limit the search for GMPs to certain areas of the premises. Decisions such as these can be taken on a case-by-case basis and can be based on pragmatic criteria as long as the search parameter is defined clearly. Obviously it should include those areas that are most likely to host GMPs.

The size and configuration of the perimeter for the objects to be surveyed should allow for the retrospective localisation of the origin of samples which may test positive for GMP in the laboratory. Similar to the monitoring of rail and road networks, the perimeter of objects or sub-units could be divided into smaller areas in which an aggregate sample of leaf material of potential GMPs is taken.

Depending on the GMP in question and the type of goods, the total number of objects constituting the survey area may be very small or very large, it may include only a few marine ports or two dozen grain mills or 150 railway stations. The 2% rule discussed in Chapter 7, according to which approximately 2% of the survey area should be sampled per annum, is particularly suited to situations where there are between 50 and several hundred objects. Where the number of objects is significantly smaller, higher annual proportions of sampled objects will be required and should generally be manageable. Moreover a less complex sampling strategy would be required. However, where there is large number of objects in the order of 500 and more, a 2% sampling rate may constitute an almost insurmountable annual workload. In such cases it is likely to be permissible, including from a perspective of statistical validity, to reduce the proportion of objects to be sampled per year.

Notes on sampling

The manner in which leaf samples are collected in the field is basically identical to that used for the railway and road network modules. The same Standard Operating Procedures (SOP; see Chapter 6) apply. Depending on the plant density, all or only a sample of the individuals present are taken into consideration.

In contrast to the monitoring alongside linear features such as railway lines or roads, there will be an area-wide search for the survey objects associated with processing facilities. Nonetheless it is reasonable to assume that spontaneous populations of GMPs will initially occur in close proximity to clearly recognisable structures such as access roads, conveyor belts, loading ramps or silos, all of which should be given preference in the search.

Sampling efforts at processing facilities should not overlook residues of cleaning and filtering processes or other processing steps. However, standard operating procedures for residue sampling have not yet been defined. Such residues may indeed contain viable plant material, as was shown in investigations of residues from cleaning durum wheat for pasta production in Switzerland (Schulze et al. 2015). Depending on the manner of disposal, such residues may introduce GMPs into the environment, e.g. in the form of organic material for composting or piles of waste dumped outdoors. Therefore it is important to find out how production waste is handled and, where necessary, to include processing residues in the sampling regime.

Summary of investigations to date

In Switzerland, processing facilities were included in GMO monitoring at an early stage. The Swiss Federal Office for the Environment (FOEN) as well as individual cantons commissioned targeted surveys of processing and transshipment facilities for spontaneous populations of GM oilseed rape and, more rarely so, for GM *Arabidopsis thaliana* (at research institutes). At the same time, the FOEN also

commissioned a systematic nationwide review of processing facilities in order to identify those facilities that are of relevance for GMO monitoring. To this end, two different approaches were employed independently:

- a) query of the Swiss commercial register;
- b) analysis of data held by the Swiss customs authorities.

In the commercial register, a step-by-step selection process progressively narrows down the number of company entries. In contrast, the analysis of the flow of goods based on customs authorities' data directly leads to the buyers of certain goods. The two reviews were conducted independently of each other. Ideally they would be combined and the results reconciled. The findings are described briefly below (Table 5). It is of note that the number of resultant addresses differs significantly between the two approaches.

a) Search by means of the commercial register
A commercial register is a database of companies or institutions carrying out commercial activities. In Switzerland it is maintained by federal and cantonal authorities and is accessible to the public. It contains all private and public establishments that engage in commercial activities. The various activities are divided into fixed categories. In Switzerland these follow the General Classification of Economic Activities (NOGA). These categories make it possible to filter the entries to include only those out of the tens of thousands listed that are of interest to monitoring undesirable populations of GMPs.

A first query of entries, using 19 of the total of 794 different NOGA classifications of economic activities, resulted in more than 100,000 company entries. Therefore the selection was further limited to 16 criteria, resulting in 2498 commercial register entries. Subsequent clarifications of the individual cases allowed for more than 85% of these entries to be discarded, leaving a total of 333 entries to which priority should be given in a monitoring of processing facilities in the context of releases of GM oilseed rape. These companies are located all across Switzerland with a focus on the Central Plateau and primarily include the following types of facilities (categories pursuant to NOGA classification):

- Manufacture of prepared feeds for farm animals (n = 42)
- Manufacture of grain mill products (n = 96)
- Freight rail transport (n = 93)
- Storage (n = 26)
- Wholesale of grain, unmanufactured tobacco, seeds and animal feeds (n = 63)
- Research and experimental development on biotechnology (n = 3)

b) Research by means of customs authorities' data:
The Swiss customs authorities maintain a database containing information on import volumes of commodities and on recipient companies. These data are a direct lead to the companies which purchase GMP-relevant imported commodities. The selection process described under a) is largely unnecessary when taking this approach. While this customs data approach would appear to be simpler and more direct, it does have some disadvantages:

- Import figures vary strongly between years. Several years' worth of data must be analysed in order to reliably identify relevant companies.

- The commodities' recipient registered with the customs authorities is not necessarily the same company as the one that ultimately processes the goods.
- The recipient's address registered with the customs authorities is not necessarily the same address as the address of the branch at which the goods are actually processed.

This means that even though the customs authorities provide good underlying data, additional clarifications and research are needed.

Data on commodity imports in 2012 to 2014 were used to identify significant processing facilities for GMO monitoring. As a first step, the methods and criteria described in Chapter 3 were used to identify commodities with a higher than normal probability of containing GMPs (Table 4). The commodity classifications were then used to filter for domestic recipients. The aggregate number of recipient companies and institutions for all types of commodities concerned is 142. Each company or institution receives at least one of the "suspect" commodities. If one disregards import quantities of less than 10 tons over the three-year period, 52 company names remain (Table 5). Evidently seeds and animal feed are often imported in small quantities.

Commodoty	Tariff-No.	Australia		China		Canada		USA	
		t/a	GMP	t/a	GMP	t/a	GMP	t/a	GMP
Foliage, grasses, mosses, lichens	0604			117	Po				
Wheat	1001					72'554	Fl, Lu, Ra	3'626	Lu, Ra
Soya	1201			1'385	Po	853	Lu, Ra		
Linseed (flaxseed)	1204			285	Po	68	Ra		
Seeds for cultivation	1209	85	Ra	72	Po	119	Fl, Lu, Ra	336	Lu, Ra, Gr
Straw and grain chaff	1213			7	Po				
Beets, hay, alfalfa and similar such feed	1214			8	Po	52	Fl, Lu, Ra		
Residues from soybean oil production	2304			9'849	Po	274	Lu, Ra		
Bird feed	2309					1	Fl, Ra	53	Ra

Table 4: List of agricultural products imported into Switzerland which have a higher than normal probability of being contaminated with GMPs. t/a = data in tonnes per year (average of years in which imports occurred in the 2012-14 period). Orange = 1. Priority for monitoring undesirable populations of GMPs, contamination is expected; Yellow = 2. Priority for monitoring undesirable populations of GMPs, clarification as to contamination recommended. GMP = contaminating species: Gr = bentgrass and other grasses, Fl = flax, Lu = lucerne (alfalfa), Po = poplar, Ra = rapeseed. Data source: Swiss-Impex, Swiss Federal Customs Administration FCA.

Customs Tariff No.	Commodity name	Number of addresses	Number of addresses >10 t
1001	Wheat and mixed grains	26	19
1201	Soyabeans	14	12
1204	Linseed	14	8
1209	Seeds for cultivation	21	12
1214	Hay	1	1
2304	Soyabean press cake	2	2
2309	Animal feed (incl. bird feed)	72	3

Table 5: Number of addresses in Switzerland receiving commodities that are the focus of a GM oilseed rape monitoring programme. The data are based on import data for 2012-2014 as issued by the Swiss Federal Customs Administration. The column on the far right only considers addresses in receipt of a total of more than 10 tons of the relevant commodities over the three-year period.

Fig. 7: Transfer of openly transported agricultural products at the Rhine Port in Basel, Switzerland (Photo credit: Dirk Hamburger).

4.5 Floristic mapping

This module describes the standardised approach to floristic mapping of spontaneously occurring GMPs, their potential counterparts for hybridisation, and potential hybrids. The approach is described in detail in the GMO monitoring standards for floristic mapping (*Richtlinie 4330 Blatt 10*, German only) published by The Association of German Engineers VDI (VDI 2011). This chapter provides an overview of its objectives and methodology. A pilot project on GM rapeseed monitoring in Germany offered the opportunity to thoroughly test this method of floristic mapping (Chapter 5). Based on the results of this research project the VDI Standard is currently being reviewed. The revised, new and binding version of the standard is scheduled for publication in late 2018 (www.vdi.de).

The mapping method described in the VDI Standard is designed for an individual, spatially isolated source of spontaneously growing GMPs. A modified methodology would be required for several sources of GMPs located in close proximity, i.e. several fields in which genetically modified crops are being cultivated.

Floristic mapping in conjunction with molecular biological methods for transgene detection (Chapter 6) allow for conclusions on the frequency and spatial distribution of GMPs and their hybrids.

Aim

1. Efficient detection of undesirable spontaneous populations of GMPs, their potential counterparts for hybridisation, and potential hybrids.
2. Conclusions on the frequency and spatial distribution of GMPs, their potential counterparts for hybridisation, and potential hybrids.

Definition of the survey area

In the case of agricultural cultivation, the area to be surveyed includes a prior GMP cultivation area as well as sites and tracks in the vicinity of current or prior GMP cultivation areas. In the case of transports, processing or the use of GMP as

animal feed, the sites associated with the processing, storage, transshipment and usage of GMP as well as sites in their vicinity including access roads and tracks are to be surveyed (*VDI RL 4330 Blatt 10*).

Structuring of the survey area
The perimeter of the survey area is to be drawn at a distance of at least 1 km around the current or prior GMP cultivation area and around sites associated with the processing, storage, transshipment and usage of GMP. Within this area, sites and tracks as well as access roads and tracks including their verges are to be surveyed.

Objects for sampling
Before floristic mapping can commence, the plant species to be mapped and the sites to be surveyed in the survey area must be determined. Whether or not the mapped plants do indeed have transgenic traits can only be determined as part of the subsequent molecular biological analysis of plant samples (Chapter 6).

Notes on sampling
Plant are sampled for molecular biological analysis in accordance with the VDI guidelines for sampling for the purposes of GMO monitoring (*Richtlinie VDI 4330 Blatt 5*, German only) or in accordance with the methodology described in *BVL G 30.10-1* as part of the official methodology collection by the German Federal Office of Consumer Protection and Food Safety (BVL) of investigation methods pursuant to Article 28b of the German Genetic Technology Act (*Gentechnikgesetz, GenTG*).

Summary of investigations to date
Floristic mapping pursuant to the VDI Standard was tested and advanced as part of a research project entitled “Development and testing of a concept for monitoring of approved transgenic rape imports under Directive 2001/18/EC: results of an R&D project (FKZ 3511 89 0100) of the Federal Office for Nature Conservation” (Wedlich et al. 2016). The research project and its results are outlined in Chapter 5.

Fig. 8: Ruderal oilseed rape in an industrial area near Mannheim, Germany (Photograph after Wedlich et al. 2016).

5 Case study: Monitoring GM oilseed rape

The previous chapter provided individual components of a GM monitoring programme, with each of the components covering a category of release locations or individual exposure pathways. However, for a complete monitoring of individual GMPs it may be necessary to combine several components. This is illustrated in Chapter 5, using case studies on GM oilseed rape monitoring in Germany and Switzerland. Moreover, these examples show that a monitoring programme for the same genetically modified plant may be designed in different ways depending on the situation at hand. This is not necessarily due to differences in the planners' preferences but rather differences in the conclusions drawn from the prior analysis of priority exposure pathways and release locations. This once again highlights the importance of thorough clarifications prior to designing the actual survey.

5.1 Concept for GM oilseed rape monitoring in Germany

The research project on monitoring of genetically modified ruderal oilseed rape in Germany (Wedlich et al. 2016) is divided into two parts. In the first part a

comprehensive investigation localised the commodity flows and potential sources of possible exposure and also locations hosting GM ruderal oilseed rape. The investigation took into consideration the cultivation regions and main transport routes of GM oilseed rape but also considered secondary importation routes. The second part of the research project consisted of the actual work that allows for the detection of GM ruderal oilseed rape. The concept was developed and tested in 2014 to 2016. It is not currently carried out routinely.

.

At the time the research project was underway, four transgenic cultivars of oilseed rape had been authorised in the European Union for importation and processing (Wedlich et al. 2016). The focus of the GM monitoring programme presented here is on these authorised cultivars of oilseed rape. Based on the main transport routes for imported GM oilseed rape, the recipient processing facilities including their wider surroundings were mapped. Targeted searches were conducted, for example, along railway tracks, roads and conveyor belts. The concept thus combined several of the approaches outlined as separate modules in Chapter 4.

Definition of the survey area

The prior investigations had shown that the most important initial transport routes for oilseed rape were waterways, leading from ports on the Rhine to the central oil mills and feed manufacturers. Other installations located in the immediate vicinity of these routes include silos, biodiesel production sites and installations owned by agricultural cooperatives. All these facilities receive a major proportion of the imported oilseed rape for further processing. Given the circumstances, the focus of the concept was on the Rhine River and the local processing facilities. In addition to the Rhine, some of its tributaries (Neckar, Lippe) were also included in the study. The study thus covered only the most significant locations for releases of GM oilseed rape. Other potential release locations such as, for example, the German international ports were not taken into consideration.

Structuring of the survey area

The manner in which seeds of oilseed rape enter the environment in the course of transshipment has been relatively well researched. The same is true for the characteristics of the locations hosting spontaneousy occurring oilseed rape plants. Based on this knowledge it was concluded that a wider area around the processing facilities would need to be surveyed for monitoring purposes. Therefore, prior to the survey an area of one kilometre around the priority sites was explored: buildings and facilities for processing or storage, transshipment or onward transport of goods including access roads. This exploration made use of satellite images, which are available online and free of charge. Following this step, it was possible to explore the locations in detail on the ground and establish definitive perimeters. Floristic mapping in accordance with the standards issued by the Association of German Engineers (VDI 2011) was carried out at the same time.

Especially the transport routes to the waterways as well as the main connecting roads were taken into consideration for floristic mapping. Following the delimitation of the survey area these sites were systematically searched for the presence of GM plants. This work yielded the following important findings:

A not insignificant number of sites considered for surveying proved to be unsuitable, as they were blocked by fences or other barriers or because they were not accessible for reasons of security.

- The accessibility of sites should be ascertained carefully beforehand.
- Authorisations for access to non-public sites such as ports or railway tracks must be obtained prior to site visits.
- Additional costs associated with the above must be calculated, e.g. accompanying security personnel.

Notes on sampling

Locations at which spontaneously growing individual oilseed rape plants or local groups of plants were found were marked on prepared maps, using dots, lines or area symbols. In addition, their GPS coordinates were taken and they were photographed. Therefore, should they test positive in the laboratory their exact locations will have been recorded. The estimated number of individuals was recorded on a log sheet. As a minimum the following information was recorded:

- a brief site description (name, street name, company, location),
- the population size (number of individuals in the local population),
- the plants' development stage (phenology),
- the size of the plants,
- the distance of the site at which the plants were found to agriculturally cultivated oilseed rape crops,
- the presence of other Brassicaceae species[13] (potential outcrossing),
- the vegetation type in which the oilseed rape plants are growing,
- any particular site characteristics that contribute to the interpretation of the findings (disturbances, herbicide use).

The oilseed rape plants found were pragmatically assigned to smaller, clearly delimited populations. Aggregate samples of leaf material were then taken of the plants in each of these populations.

It is important to not only record the locations at which oilseed rape plants were found, but also to document the entire mapped area or surveyed transect, so as to retain a record of where searches have have not been conducted, since it is hardly possible to achieve full coverage of very large sites (Fig. 9).

Leaf samples were taken of the individual oilseed rape plants for laboratory analysis. Where the local populations of oilseed rape consisted of less than 30 plants, all individuals were sampled. The samples were combined into aggregate samples or bagged individually. Where local oilseed rape populations consisted of more than 30 plants, leaf samples were taken only from a random sample of individual plants.

The monitoring concept provides for multiple site visits for sampling in a given year. Ruderal oilseed rape flowers over long periods and new populations may flower for a second time following an initial flowering phase. The most important time for fieldwork is the main flowering period in the springtime. A second flowering

[13] An identification key for closely related Brassicaceae species was developed to allow for the detection of potential counterparts for hybridisation of oilseed rape. See Annex 3 in Wedlich et al. (2016).

phase may occur in late summer. This can be an expedient time for a second field survey.

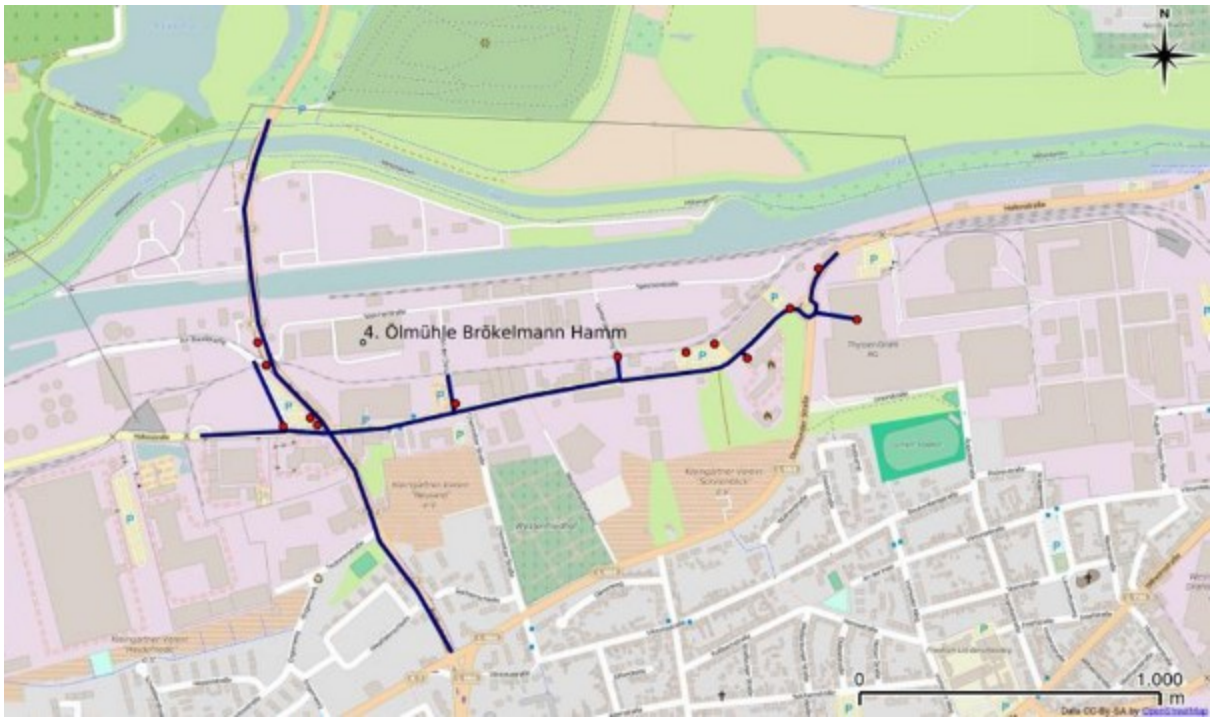


Fig. 9: Sampling locations (red dots) including survey tracks (tracks in dark blue) in Hamm near to the oil mill of Brökelmann Co. Ölmühle GmbH & Co.. The road, rail and waterway networks are also visible. By no means all the access roads and railway tracks were actually surveyed. Plan produced in QGis 2.2.0 using Openstreetmaps as the base map. After Wedlich et al. (2016).

Summary of investigations to date

The monitoring concept for spontaneous populations of GM oilseed rape was tested in 2014 when initial survey data were obtained. A total of 17 locations along the waterways were sampled. These included nine oil mills, seven livestock feed manufacturers and one railway station. Due to the often observed populations of oilseed rape along the motorways, two motorway rest stops were included in the sampling regime in April 2014.

Sampling of oilseed rape (*Brassica napus* L.) was conducted from the end of April to the end of May 2014. A total of 136 local oilseed rape populations was recorded and sampled. An additional eight populations of arugula (*Eruca sativa*) as well as one population of charlock (*Sinapis arvensis*) were also recorded. Both of these species are close taxonomic relatives in the Brassica family. The oilseed rape populations occurred in a variety of locations, primarily along roads and railway tracks, but also on riverbanks, at roundabouts, on motorway approaches and on actual company premises. In many instances, however, the latter were not accessible for sampling.

Based on the frequency distribution of population sizes for all sites, the following picture emerges:

- Oilseed rape populations most frequently comprised between six and 25 individual plants. Populations of this size comprised approximately 50% of all populations (Fig. 10).

- A further 20% of the populations comprised between 26 and 50 individual plants.
- Only sporadic populations were very large, consisting of between 250 and 500 individual plants.

Out of the 136 tested oilseed rape populations only a single sample proved to be a transgenic. The relevant sample had already been detected by immunoassay in the rapid test, after which all 10 leaves comprising the aggregate sample were tested individually. In this repeat test only a single leaf tested positive. PCR analyses conducted by two independent laboratories later confirmed the suspicion of herbicide tolerance conferred by genetic engineering (Franzaring et al. 2016).

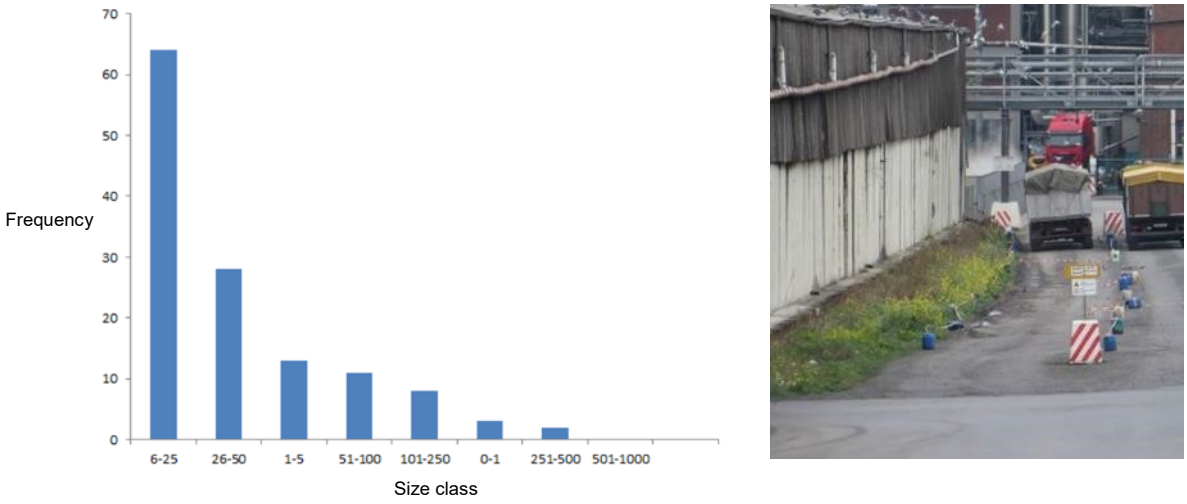


Fig. 10: Left: Frequency distribution of population sizes of local occurrences of ruderal oilseed rape. Most frequently, oilseed rape populations were small. Right: large population of ruderal oilseed rape at a company premises. Images by Wedlich et al. (2016).

5.2 Oilseed rape monitoring in Switzerland

In Switzerland, the commercial cultivation of GM oilseed rape will continue to be prohibited at least until 2021. While the importation of feedstuffs containing GM oilseed rape is legally permitted, currently there are no such importations. Therefore the Swiss situation differs from the German situation in that in Switzerland the focus of the monitoring of undesirable populations of GMPs is not on authorised imports of oilseed rape but rather on undeclared GMP components or inadvertent contaminations with GM oilseed rape. The discoveries to date of spontaneous GM oilseed rape populations in Switzerland are ascribed to Canadian durum wheat imports which do indeed contain GM oilseed rape (Schulze et al. 2015). Another factor is the dispersal of contaminated bird feed (Schoenenberger et al. 2016). All imported goods which may be contaminated with GM oilseed rape are therefore of significance. This means that a broad range of commodities, transshipment facilities and transport routes must be considered for monitoring and initially the priorities are not very apparent. Given that the territory of Switzerland is small compared to Germany, a monitoring concept with a broader coverage of potential release locations would appear to be feasible.

The Swiss monitoring programme for spontaneous GM oilseed rape populations is composed of modules as described in Chapter 4:

1. Processing and transshipment facilities: This component constitutes targeted monitoring of those companies and facilities that are most likely to be sources of the entry of GM oilseed rape into the environment (Chapter 4.4). Examples would be transshipment facilities for imported goods as well as their surroundings and access tracks. This module also covers locations at which birds are regularly fed, since feed mixes for birds regularly contain seeds of GM oilseed rape (see Chapter 4.3). The selection of sites to be monitored is based on analyses of commodity flows and is continuously revised based on the latest findings. This module has been in existence since 2014. Residues and processing wastes are not yet subjected to routine surveys.

2. Railway network: The discovery of GM oilseed rape in the vicinity of railway tracks gave rise to the question as to a potentially wider distribution of GM oilseed rape along the railway network. Since 2011 randomised searches along the railway network have sought to answer that question. Every year, a total of approximately 30 km of railway track is surveyed as part of the monitoring programme (Chapter 4.1).

3. Road network: Lorries account for the finer distribution of goods potentially containing GMO as well as for a large proportion of the transit of goods. Similar to the railway network, the aim would be to conduct random surveys to ascertain as to whether these transports are a source of the establishment of GM oilseed rape in the wild. The monitoring concept for the road network is still under development. Trial surveys were conducted in 2014 and 2015 (Chapter 4.2). Routine surveys are not as yet being conducted.

4. Seed, food and feed: Seed and feedstuffs are distributed in the environment in a targeted manner. Even very minor admixtures of GM material may give rise to the successful establishment of oilseed rape plants. Quality controls for seed therefore tend to be very thorough. In contrast to the other modules which are under the aegis of the Swiss Federal Office for the Environment FOEN, the responsibility for seed lies with the Swiss Federal Office for Agriculture FOAG while the Federal Food Safety and Veterinary Office FSVO is in charge of food. Imported shipments of arable crop seed are routinely tested. Similar controls for seed for meadows and lawns are under development. Feedstuffs are also tested for traces of genetically modified organisms. There are still gaps in this area, for example when it comes to bird feed or with regard to impurities consisting of GMPs other than the commodity in question (Chapter 4.3).

6 Methods: Sampling and analysis

The detection of transgenes in a sample of plant material is the final step in a series of clarifications and surveys used to confirm the presence of undesirable populations of GMPs in the wild. Sampling and laboratory analysis are particularly complex and place great demands on the diligence of staff performing this work and on the quality of materials and appliances. Highly standardised procedures

are required in order to ensure high and consistent sensitivity for transgene detection. In laboratory practice, these procedures are known as “Standard Operating Procedures” (SOPs).

As a basic principle, both the sampling of plant material and its analysis must be undertaken in a manner that is adapted to the plant species in question. However, species-specific SOPs are available only for very few GMPs. The bulk of the existing experience in Europe relates to the detection of transgenes of GM oilseed rape cultivars.

However, for GM oilseed rape there is not just a single SOP for sampling and GM detection. Different SOPs are in use in Germany and Switzerland respectively. It is reasonable to assume that other countries have also developed SOPs.

6.1 Taking samples in the field

A possible Standard Operating Procedure for taking leaf samples of oilseed rape is provided In the Annex to this report. It describes the procedure used in Switzerland. The document is in the public domain and is available free of charge. The SOPs used in Germany have also been published.

- Switzerland: “SOP for the sampling of rapeseed for laboratory analysis” in the version dated 18 May 2017; published online by the Swiss Federal Office for the Environment (FOEN)[14]. Available for download free of charge.
- Germany: “*Probenahme von Pflanzenmaterial*” (Sampling of plant material). *Methodensammlung des Bundesamtes für Verbraucherschutz und Lebensmittelsicherheit, G 30.10-1* (Methodology collection published by the German Federal Office of Consumer Protection and Food Safety; German only). Pay-walled access at www.methodensammlung-bvl.de .
- Germany: “*Monitoring der Wirkungen gentechnisch veränderter Organismen (GVO) - Leitfaden zur Entnahme und Aufarbeitung von Pflanzenproben für die molekularbiologische Analytik”. Richtlinie VDI 4330 Blatt 5*. (Monitoring the effects of genetically modified organisms (GMOs) - Guidelines for sampling and processing plant samples for molecular biological analytics. VDI 4330 Sheet 5; this sheet has not yet been translated into English.) Pay-walled access at www.vdi.de .

Other SOPs for other plant species are largely congruent with those for oilseed rape, for example the SOP published in Switzerland in 2017 for the sampling of leaf material of alfalfa (*Medicago sativa*). The Swiss SOPs for oilseed rape and alfalfa are practically identical apart from the timing of sampling. The SOP for alfalfa can also be obtained free of charge from the FOEN website.

6.2 Analysing samples in the laboratory

Rapid tests for rapid results?

The samples of plant material collected in the field are normally only tested in the laboratory, using molecular genetic tests. Event-specific detection using real-time

[14] https://www.bafu.admin.ch/bafu/en/home/topics/biotechnology/info-specialists/monitoring-gentechnisch-veraenderter-organismen/grundlagen-und-praktische-empfehlungen-fuer-den-umgang-mit-gente.html

polymerase chain reaction (PCR) is indispensable. If it is useful to gain initial indications of the presence of GMPs prior to this analysis, rapid tests may be used. While these rapid tests cannot replace PCR, they can quickly and reliably indicate which of the samples contain GM plant material. This allows for a more targeted use of the more elaborate PCR analysis.

For rapid testing, the plant material is homogenised in a mortar and mixed with extraction buffer. In the presence of GM plant material the solution results in the test strip taking on a characteristic coloration. However, the test strip coloration is not based on DNA quality[15] but is caused by characteristic proteins contained in the solution in combination with antibodies on the strip (immunoassay). Various companies offer a range of different products which can be used specifically for certain transgenes (e.g. cry1A, cp4epsps, pat etc.). It is important to note that:

- A single rapid test may not cover all GM lines of a particular plant. Therefore it is important to at first identify which of the GM lines might be present and which of the rapid test products are suited to detecting these lines.
- The manufacturers' indications of the rapid tests' sensitivity are generally only applicable under ideal conditions in a laboratory (homogenisation of sampled material, handling of samples). Their sensitivity is lower under field conditions.
- In the field, where there is no suitable workspace, the use of rapid tests is practicable primarily for samples taken from individual plants.

Standard procedures for laboratory analysis

Laboratory analysis of prepared plant samples using molecular genetic methods is complex and must be conducted by an experienced laboratory. These analyses can only be conducted if the transgenic DNA sequences are known and the event-specific DNA markers have been developed. In the EU, GMP approval is conditional upon detection methods being available.

The event-specific laboratory analysis is comprised of a number of steps: Crushing of the leaf material, homogenisation of the leaf material, DNA extraction, and conducting the real-time PCR. These and other steps of the work process are conducted in accordance with their own specific SOPs. The details will not be dealt with in this technical report. They are a matter of the relevant laboratories' expertise and responsibility. Technical guidelines can be found in the methodology collection published by the German Federal Office of Consumer Protection and Food Safety (*BVL G 30.40-1 bis G 30.40-17*; German only; pay-walled access at www.methodensammlung-bvl.de).

Sensitivity of methods

It is inefficient to test individual specimens where there are larger populations of potential GMPs. Therefore, aggregate samples containing material collected from several plants are tested simultaneously. This of course immediately leads to the question as to how many samples may be aggregated for a single GMP to still be detectable.

[15] Recently, rapid test products have entered the market and they react directly to the presence of typical DNA sequences. See, for example, www.envirologix.com/gmo-testing/gmo-testing-kits-dna/.

For GM oilseed rape, findings of a comparison between real-time PCR and ELISA rapid tests are available from Switzerland. These tests were conducted by an independent laboratory (KLBS 2017) and were prompted by diverging analysis results for rapeseed contained in bird seed using ELISA rapid tests and real-time PCR respectively. The most significant findings are as follows:

- The detectability threshold for the transgene differs between the two methods (rapid test and real-time PCR respectively). The detectability threshold was higher by a factor of between 5 and 20 when using rapid tests compared to real-time PCR (Table 6).
- The different qualities of the samples used (fresh leaves or desiccated leaves respectively) did not impact on the sensitivity of the two detection methods. When the samples consisted of seeds, the detectability threshold was minimally higher (0.1%) for PCR.
- Both detection methods would appear to be sufficiently sensitive, at least under laboratory conditions, to detect one GMP leaf sample among 50 leaf samples as part of an aggregate sample (approximately 1 cm² per individual sample). Both methods could even test as many as 100 leaf samples simultaneously. However, aggregate samples consisting of more than 50 individual samples have proven inefficient for laboratory work.
- For sites at which the occurrence of GM oilseed rape is not very likely, the recommendation is to analyse aggregate samples of up to 50 individual leaf samples, both for real-time PCR and ELISA rapid tests. Caution: Only when using real-time PCR can a potential inhibition of the analytic reaction (a technical "glitch" at the lab) be controlled. With rapid tests it remains undetected.
- For sites at which there is a heightened probability of the occurrence of GM oilseed rape, the aggregate samples should consist of no more than 10 individual leaf samples or seeds respectively for reasons of efficiency. Positive results obtained with rapid tests must always be confirmed using real-time PCR.

Analysis method	**"Direct" leaves**	**"Desiccated" leaves**	**Seeds**
real-time PCR (confirmation of GT73 event)	0.05% (1:2000)	0.05% (1:2000)*	0.1% (1:1000)
ELISA rapid test (detection of CP4 EPSPS)	0.5% (1:200)	1% (1:100)*	0.5% (1:200)

Table 6: Detectability thresholds for two different methods and three different qualities of plant material samples of GM oilseed rape (GT73 Roundup Ready® by Monsanto) under laboratory conditions. "Direct" leaves = taken immediately post-harvest from intact plant. *=highest tested dilution. Therefore the detectability threshold is presumably even lower. Table from KLBS (2017).

7 Sample design

If the survey area is very large (e.g. the reference area is the whole of Switzerland), a full-coverage search will not be feasible. Even if the focus is confined to specific areas such as the road or railway network, comprehensive monitoring is still an extremely elaborate and time-consuming process. It is therefore necessary to identify a strategy that can yield maximally precise statements about the entire survey area on the basis of partial data collection (sampling).

7.1 Three strategies: A schematic overview

A suitable survey design is one that enables accurate and precise conclusions about the entire survey area to be drawn from the sample data. Three sampling strategies based on the monitoring of spontaneous populations of genetically modified plants (GMPs) along transport routes are outlined here. The diagram below depicts the initial situation in a hypothetical pilot study of the incidence of spontaneously growing GMPs (Fig. 11).

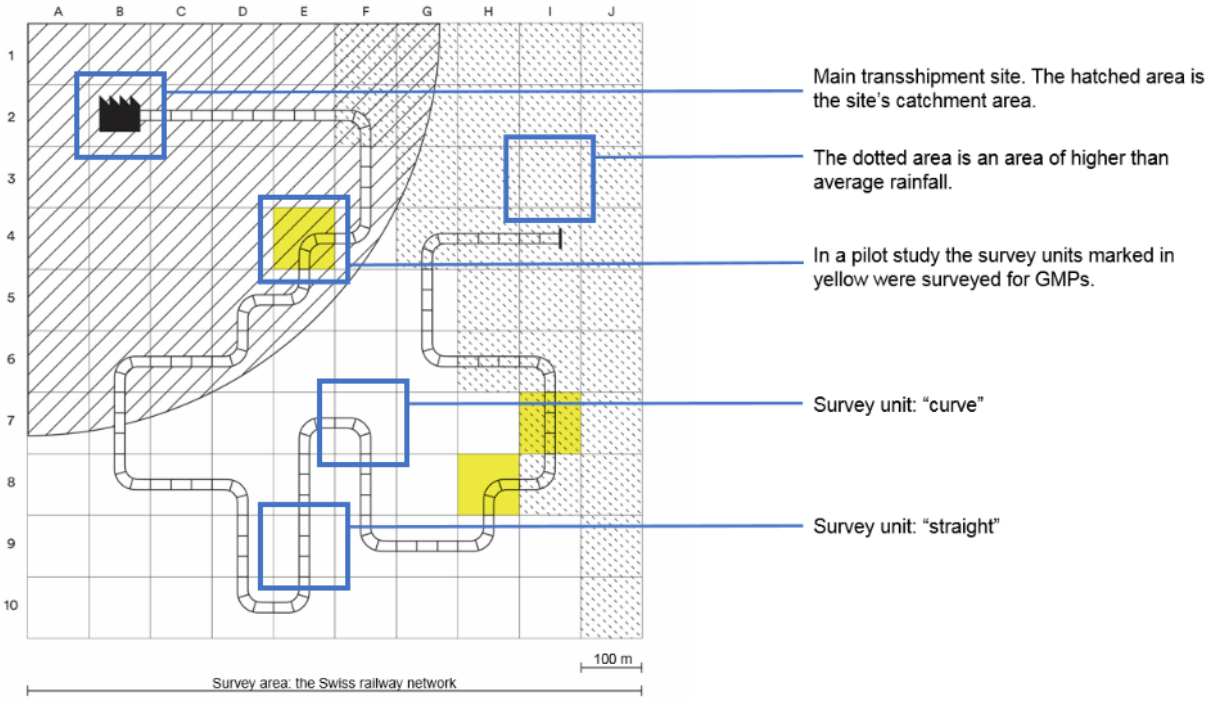
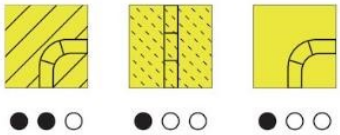


Fig. 11: Schematic representation of the initial situation. The survey area is divided into sampling units of 100x100 m. One section of the survey area is close to a transshipment site; another section is in an area in which precipitation is higher than average. A (hypothetical) pilot study involved looking for spontaneous populations of genetically modified plants (GMPs) in three randomly selected survey units (yellow) along the railway network. Diagrams: *yaay.ch*.

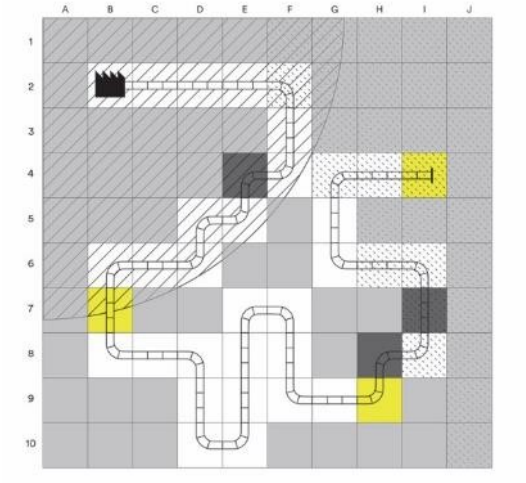
As a result of the hypothetical pilot study, it is now known that spontaneously growing GMPs occur more frequently on curves close to the transshipment site. By contrast, on straight stretches in a high-rainfall area and on curves in relatively dry areas the incidence of GMPs is average. The results of the pilot study can be summarised as follows (the larger the number of black dots, the higher the probability that GMPs will occur):



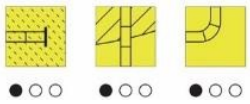
However, there is not yet any data on straight stretches of track in dry areas, although the findings so far indicate that spontaneous GMPs only rarely occur here.

The question that now needs to be answered is whether and how the information from the pilot study can be used in designing the sampling method for the future monitoring programme. Traditional survey designs require samples to be taken at random. This means that the information from the pilot study cannot be used (Strategy A). Purely random sampling is not suitable if data on very rare events needs to be collected – such as data on the spontaneous occurrence of GMPs as a result of transport losses.

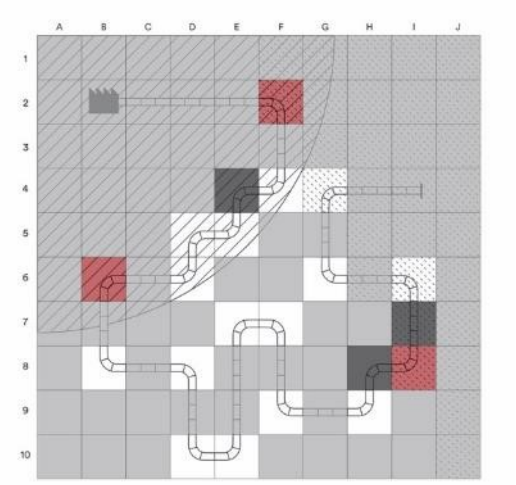
A more effective way of detecting GMPs is to focus the search either on areas in which the pilot study indicates that GMPs are more likely to occur (Strategy B) or on areas where little is yet known about the occurrence of GMPs (Strategy C). The diagram on the next page compares the three strategies.



Strategy A: Random sample
In traditional sampling methods the probability of being selected for the sample (“selection probability”) is the same for all survey units. This means that the probability that GMPs will occur in the areas that are surveyed is only average:

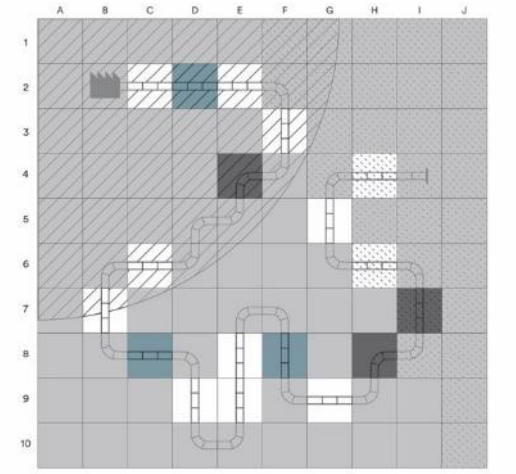


Occasional and clustered events are poorly captured: they tend to “slip through the net” of the measurement grid.

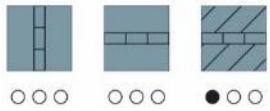


Strategy B: Maximise the number of "hits"
This strategy aims to maximise the number of GMP occurrences that are detected. Data collection therefore focuses on survey units containing curves (white areas). In all the areas surveyed the probability that spontaneous GMP populations will occur is above average:

This strategy is particularly appropriate if the localised occurrences are subsequently to be controlled.



Strategy C: Close knowledge gaps
The aim here is to close knowledge gaps in order to arrive at the best possible estimate of the number of GMP populations in the survey area as a whole. In order to supplement the information from the pilot study in the most useful way, surveying now focuses on the straight stretches (white areas). The results will potentially confirm that GMPs occur only rarely along these stretches:



This strategy is appropriate if the number of GMPs in a survey area needs to be estimated as accurately as possible.

7.2 Capturing rare events: Statistical principles

Traditional sampling methods are not ideal
Many traditional statistical estimation methods require the sample to be drawn largely randomly from the set of all potential survey units (Strategy A). Randomised sampling is therefore used in many environmental monitoring programmes (such as pollutant monitoring programmes or biodiversity monitoring). There are various random selection methods, including systematic random sampling (involving regularly distributed samples with a random origin) and stratified random sampling (involving randomly selected samples from different sections [strata] of the survey area). A feature of these traditional sampling methods is that the probability of being included in the sample (the selection probability) is similar for all survey units.

However, traditional sampling methods are of limited usefulness for capturing rare events and/or events with a clustered distribution. A clustered distribution means that events may be locally frequent but this concentration of events occurs in only

a few survey units (see e.g. the distribution of ruderal oilseed rape alongside roads in Chapter 4.2). Clustered events are therefore just as difficult to capture as rare events: purely random selection over the entire survey area may result in the target event not being recorded at all, because it is too rare and may therefore "slip through the net" of the measurement grid (Guisan et al. 2006). Experience to date shows that GMPs a) occur only rarely and b) are more frequent in the vicinity of certain sites such as goods transshipment areas. Under these conditions, purely randomised selection of the sample over a large survey area is not conducive to the detection of GMPs. To detect GMPs in the environment using this method, a disproportionately dense measurement grid would be needed (Fig. 12).

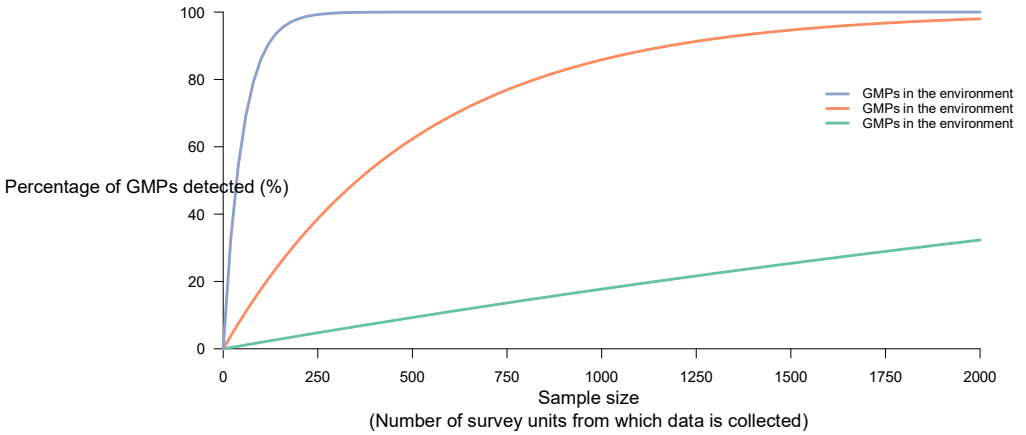


Fig. 12: The probability of detecting the occurrence of GMPs in the environment in relation to sample size. In this example we assume that the total length of the Swiss railway network is 5124 km (www.bfs.admin.ch). This railway network is subdivided into survey units 100 m in length. A variable number of these survey units are searched for GMPs. If only 10 GMPs occur along the entire railway network, the probability of finding at least one of these GMPs is less than 0.4, even if 2000 survey units are investigated.

A more efficient method concentrates the search for GMPs on the survey units in which GMPs are more likely to occur (Strategy B). This means that the selection probabilities of the various survey units are no longer equal, as they are in traditional sampling methods, but instead vary considerably. This is referred to in the literature as an "unequal probability survey design" (Brown et al. 2013). Various statistical methods can handle survey designs of this sort by weighting the data of survey units with a low selection probability more heavily than that of survey units with a high selection probability. Where possible, data collection should therefore be planned in such a way that the selection probability of each sample area is known.

Before the first data collection round it is probably not yet clear where GMPs are most likely to occur. If no information on this point is available, the first data collection round will still need to involve a random selection of survey units (this corresponds to the hypothetical pilot study in Chapter 7.1). Once the first finds of GMPs have been made, it will be easier to identify what determines their occurrence. Sampling can then focus on survey units with a higher than average probability of GMP occurrence (Strategy B) or on sites with knowledge gaps (Strategy C). If data are collected over a relatively long period or in several stages,

it makes sense to optimise sample selection between each stage. This is termed “adaptive sampling” (Thompson & Seber 1996).

In fact many different forms of adaptive sampling are described in the literature (Edwards et al. 2005, Guisan et al. 2006, Brown et al. 2013). Such methods have proved very useful for rare and clustered events – a category into which spontaneously occurring GMPs are likely to fall. They are far more efficient than traditional sampling methods (Brown et al. 2013).

Important: What is the aim of the monitoring programme?
Where monitoring is required by law, the objective is often expressed in relatively general terms (Chapter 1): the occurrence of GMPs in the wild must be monitored. Such a definition leaves some room for interpretation with regard to exactly what is needed. To avoid misunderstandings, the aim of the monitoring programme must be specified more precisely. In monitoring of the type discussed in this report, the emphasis is on the following two objectives, each of which calls for a slightly different survey design.

“Find populations”:
The aim is to find as many GMP populations as possible. In this case monitoring should involve an efficient strategy for localising GMPs that can then be controlled. Where this is the aim, the survey units selected should be those with the highest probability of GMP occurrence (Strategy B).

“Estimate the total population”:
The aim is to estimate the size of the GMP population in the entire survey area as accurately as possible and depict its trend over time. This is the classic aim of a biodiversity monitoring programme. It involves selecting survey units primarily from those parts of the survey area on which only imprecise information is available (Strategy C). The aim of adaptive sampling is thus to maximise not the number of GMP finds but the precision of the estimate of the total population. This is an essential preliminary to documenting the decrease or increase in GMPs over time. For example, this method could be used to demonstrate the success (or failure) of control measures.

What questions should the survey design answer?

What is the survey area?
The survey area should be unambiguously defined on the basis of clear criteria.

What are the survey units?
The survey area should be completely subdivided into survey units (potential measurement areas) that are as uniform as possible. For example, a survey unit could be a 100-m long section of the railway network or a square kilometre of the entire area of the country.

How many survey units should be analysed?
The number of survey units depends on the available resources (funds, personnel, time). It is important to commence by performing a data simulation in order to verify whether the amount of effort that is feasible can be expected to yield a

satisfactory (robust) result. This applies to all three of the sampling strategies that have been described.

How are the survey units selected?
As mentioned above, preference should be given to survey units in which GMPs are particularly likely to occur. Because knowledge of the occurrence of GMPs improves over the course of the data collection process, what is needed is a flexible (adaptive) survey design that can adjust to a state of greater knowledge. However, if the GMPs are so rare that no occurrence is recorded, there is no advantage in an adaptive design. In this case the prediction of GMP occurrence cannot be improved. Then again, adaptive design is also not disadvantageous, because in this case use of randomised sampling can be continued until the first GMPs are discovered.

What parameters of the total population of GMPs should be recorded?
Possible parameters are:
- the total number of GMPs in the survey area
- the number of locations with (groups of) GMPs
- the presence/absence of GMPs in each survey unit (measurement area)
- the change in the probability of occurrence of GMPs (in relation to all survey units in which no control takes place)
- the re-emergence of GMPs in survey units in which they have been controlled
- the distribution of GMPs in the survey area ("distribution map")

The same survey strategy can also be used to record hybrids with GMPs or related wild plants. The choice of parameters depends on the decisions that are to be made on the basis of the resulting facts. It is always useful to picture the main product of the monitoring programme (e.g. a diagram or table) as precisely as possible at the outset.

7.3 General rules for adaptive survey design

This chapter uses a simple data simulation to highlight the advantages of adaptive survey design and provide a basis for a couple of rules of thumb. Calculation of the potential population of GMPs in the survey area involves statistical methods developed in connection with the production of distribution maps ("species distribution modelling", Guisan et al. 2013). In adaptive survey design preference is given to survey units in which GMPs are particularly likely to occur. The method is based on a principle proposed by Guisan et al. (2006) for recording rare animal and plant species.

Scenario
Let us assume that the aim is to study the occurrence of GMPs along the Swiss railway network (5124 km, www.bfs.admin.ch). The railway network is to be divided for this purpose into survey units 100 m in length. Let us also assume that the number of GMPs (individual plants or small local groups) in a survey unit depends on three environmental factors. Firstly, the number of GMPs decreases with increasing distance from the main transshipment site for GMPs. Secondly, the number increases with the amount of precipitation. And thirdly, survey units with curves in the railway network have more GMPs than survey units without

curves. However, at the start of the study these correlations between GMP populations and environmental factors are not known. In our scenario, GMPs occur only very rarely along the Swiss railway network: 99.5% of the survey units contain no GMPs, and the remaining units usually contain one or at most two GMPs. In a simulation this hypothetical scenario results in a total population of 275 GMPs in the survey area (individual plants or small local groups).

Three sampling strategies

Because no data on the distribution of GMPs in the Swiss railway network is available at the start of the study, the plan is to start by searching for GMPs in a randomly selected 2% of the survey units – in other words, in 1024 100-m long stretches.

Before the second field period, these data are analysed, which means that the effect of environmental factors on the occurrence of GMPs is now known. This information can be used to predict the potential occurrence of GMPs in all the survey units that have not yet been sampled. Of course this works best if GMPs were actually found during the first field period. But even if no GMPs were found, it is still possible to identify areas in which the probability of occurrence is low (i.e. places with a large number of sample areas with no plants at all). The second field period now begins, in which a further 2% of the survey units are to be searched for GMPs. For this second field period, three ways of selecting the survey areas are now compared:

(A) Random sample: For the second field period the survey units are again selected purely at random.

(B) Check largest potential occurrences: For the second field period the researchers select the 2% of survey units that are predicted to have the highest probability of the occurrence of GMPs.

(C) Close knowledge gaps: For the second field period preference is given to survey units that at present have the most imprecise estimate of GMP occurrence.

Results

When survey units with the highest probability of GMP occurrence were selected, three times as many GMPs were detected in comparison to random selection (Fig. 13). Even focusing on survey units for which little information is available (Strategy C) results in the detection of twice as many GMPs as purely random selection. This contrasts with the schematic representation of Strategy C in Chapter 7.1, in which the survey units selected on the basis of this strategy had the lowest probability of GMP occurrence. The reason for this is that the random selection of survey areas in the first sampling round combined with the rarity of GMPs meant that the majority of survey units studied contained no GMPs. There is thus a knowledge gap mainly in relation to areas with elevated occurrence of GMPs. In Chapter 7.1, by contrast, the knowledge gap related to areas with low GMP occurrence (dry areas and straight stretches of track).

It is clear that a well-chosen adaptive survey design can increase the number of GMPs detected or the accuracy of the estimate of the total GMP population. One

or other of the two versions of adaptive survey design will be preferable, depending on the main aim of the monitoring programme (see above).

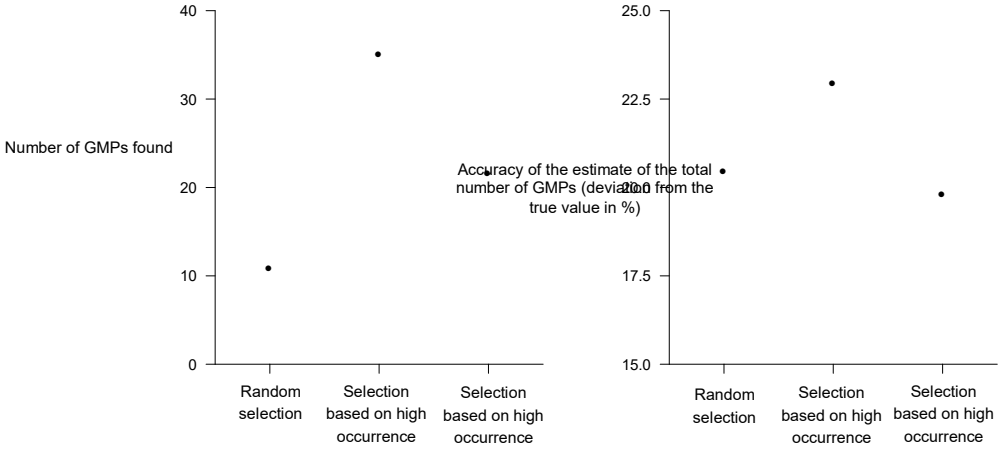


Fig. 13: The number of GMPs found (left) and the accuracy of the estimate of the total GMP population (right) vary depending of which of the three strategies is used to select the sample: random selection of survey units, focus on survey units with a high probability of GMP occurrence or focus on survey units with an imprecise estimate of the probability of GMP occurrence. The diagrams show the mean of 100 data collection simulations. The results also involve an element of luck, depending on whether GMPs are actually found in the first year of the monitoring programme.

The way in which adaptive monitoring works becomes particularly clear if GMPs occur even more rarely than was previously assumed. In Fig. 14 it is assumed that only 37 GMPs occur along the Swiss railway network. Each year 1024 survey units are searched for GMPs; this corresponds to 102 km or 2% of the total survey area. As part of the adaptive design, all the available data are analysed annually. As a result, the prediction of the distribution of GMPs improves steadily, especially in the early years. The advantages of the adaptive design are particularly clear when contrasted with the random selection of survey units (Fig. 14)

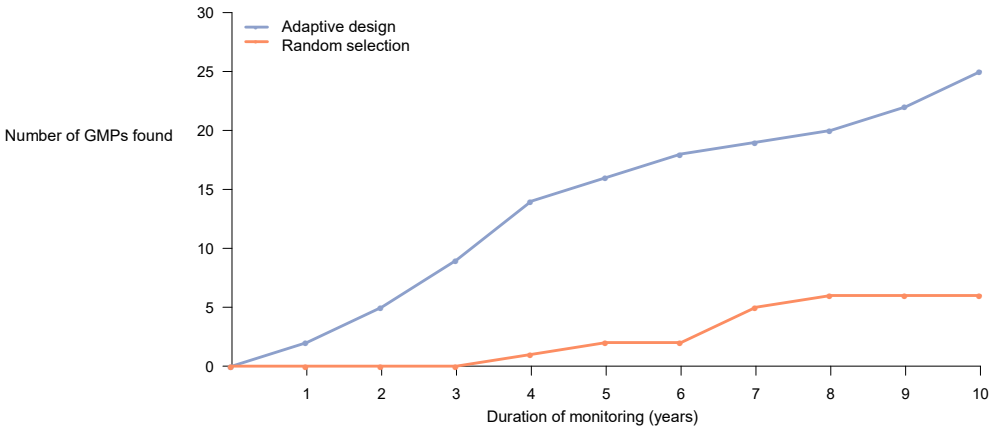


Fig. 14: Over the course of the monitoring programme, the number of GMPs found increases more with an adaptive design then it does when the survey units are selected at random. After 10 years, the adaptive design has found 25 of the 37 GMPs. This is 68% of the GMPs, despite the fact that after 10 years only 20% of the total area has been surveyed. With random selection six GMPs (16%) are found within 10 years.

General rules

Which species are to be surveyed?

The advantages of an adaptive design only bear fruit if a single species or several species with similar biology are surveyed. If the correlation between GMPs and locational factors varies for different plant species, the optimum survey design for these species will also vary. This means that if the species are to be surveyed in the same survey areas, the design of the survey cannot be optimised to the same extent for all species. This is one of the reasons why randomised sampling methods are the best choice for biodiversity monitoring programmes that involve a number of species. When monitoring individual, rare target species, on the other hand, use of a purely randomised strategy is questionable.

Sample size

The size of the sample needed to obtain data of reasonable quality depends in part on the frequency of the GMPs, their distribution within the survey area and in particular on the environmental factors that determine the occurrence of GMPs. It is therefore difficult to lay down a general rule, and any such rule should be applied with caution. A reasonable goal in our view is that annual monitoring should detect 50% of the GMP population within 10 years. In a scenario in which GMPs are very rare (in the simulation: 37 GMPs along 5124 km of the railway network) but there are environmental variables that enable the occurrence of GMPs in each survey unit to be predicted relatively well, about 2% of the survey area should be sampled annually in order to achieve this goal (Fig. 15). This 2% should be regarded as a rough rule of thumb: if GMPs are thought to be even rarer, the area that is surveyed must be increased. If the survey area is very extensive, a result of similar robustness to that in our example can be achieved from a percentage that is less than 2%.

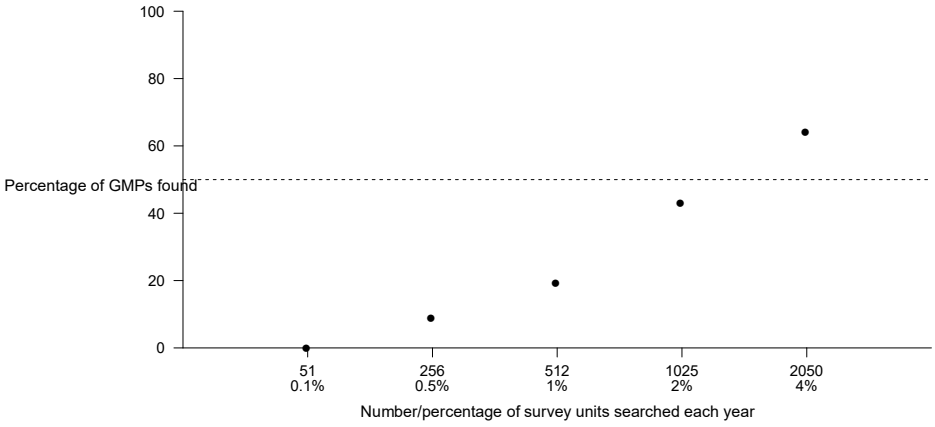


Fig. 15: The percentage of GMPs found after 10 years of monitoring. The dotted line marks the goal of finding roughly half of the original GMP population within 10 years. This involves searching for GMPs in about 2% of the survey units each year. This estimate is based on the assumption that GMPs are very rare (37 GMPs in 5124 km) but that there are environmental variables (e.g. precipitation, curved/straight stretches, closeness to transshipment site) that predict the occurrence of GMPs in each survey unit relatively well.

Size of the survey units

A key component of an adaptive survey design is the availability of data on environmental variables covering the entire survey area. However, if the survey

units are too big, this blurs the correlation between the environmental variables and the occurrence of GMPs. For example, if survey units on the railway network are several kilometres long, many will contain both straight stretches of track and curves or points, so that the correlation between the presence of curves and the presence of GMPs will be very hard to recognise. But neither can the survey units be very small, because this increases the number of measurement points and results in a lot of time being spent moving between different survey units. In our view an appropriate size for a survey unit is between 100 m and 1 km (for a linear survey area) or between 1 ha and 1 km^2 (for an areal survey area). Part of the reason for this is that important nationwide spatial data (e.g. Arealstatistik Schweiz) is available in this resolution. In addition, finds based on relatively small survey units will usually be sufficiently precisely documented to localise a population if GM plants are found in the aggregate sample from the section. Another important aspect of the optimum survey unit size is of course the effort involved in getting from one survey area to the next. If a large amount of effort is involved, larger survey units should be used. For greater efficiency it is also worth considering having several smaller survey units next to each other (clustered).

7.4 Digressions

Species distribution vs. GMP distribution
Let us suppose that we are interested in the distribution of GM oilseed rape in a survey area. This distribution depends to some extent on the distribution of oilseed rape (regardless of whether it is GM or not) – because where there is no oilseed rape, there cannot be any GM oilseed rape either. A good knowledge of the distribution of oilseed rape, including the underlying environmental factors, can therefore contribute to a better understanding of the distribution of GM oilseed rape.

Rather than simply taking a random sample of the oilseed rape plants growing in the wild in order to determine in the laboratory whether these plants include GMPs, it may therefore be useful to count the total number of oilseed rape plants. This makes it possible to investigate whether the total number of oilseed rape plants correlates with particular environmental variables. In the schematic example in Chapter 7.1, there may be more oilseed rape plants in the area with relatively high rainfall than in the dry area. More oilseed rape plants may mean more GM oilseed rape. On the other hand, the number of oilseed rape plants may not depend on the distance from the transshipment site, because it may be more heavily influenced by whether oilseed rape is being grown in the vicinity. These correlations can help improve prediction of the occurrence of GM oilseed rape.

For example, the number of oilseed rape plants could be described using a linear model based on a Poisson distribution, while the percentage of GM oilseed rape could be described with a linear model based on a binomial distribution. If the two models are combined, the result is a hierarchical model. The use of hierarchical models is becoming increasingly popular in connection with ecological questions; such models help to improve understanding of the underlying processes (Royle & Dorazio 2008; Kéry & Royle 2016). Using a hierarchical model has various advantages. Firstly, it describes the occurrence of GMPs more realistically, because it distinguishes between the two processes (occurrence of oilseed rape and the percentage of all oilseed rape plants that are GMPs). This enables the

system to be better understood and thus improves the prediction of GM occurrence. Secondly, a hierarchical model can improve the efficiency of survey design by enabling surveys of sampling areas without oilseed rape plants to be avoided.

Detection probability

So far it has been assumed that all the GMPs present in a sampled survey unit are detected. However, it is entirely possible for a survey to fail to detect some or even all of the GMPs that are present because they are too small and concealed by other plants. Alternatively a survey unit may contain a very large number of oilseed rape plants and it may not be possible to analyse all of them in the laboratory: this can also result in some GMPs not being detected. In such situations the detection probability is therefore less than 100%. Particularly if the aim is to estimate the total population of GMPs, it is important to know what the actual detection probability is and to take this into account if necessary when estimating the total population. If this is not done, the total population may be significantly underestimated.

There are a number of ways in which the detection probability can be taken into account when estimating the total population. Many of these methods – including distance sampling, capture-recapture methods, occupancy models and n-mixture models – have been developed for the purpose of recording mobile organisms (MacKenzie et al. 2002; Kéry & Royle 2016). However, there are now many studies that show that these methods should also be used with plants and that a detection probability of 100% cannot be assumed for plants (Chen et al. 2013).

If the above-mentioned methods are to be used, the data that is collected must meet certain criteria. For example, in the case of distance sampling the distance between the GMP and the recorder must be noted. In the case of other methods, at least some of the survey units must be surveyed more than once (independently). It is therefore important to decide at an early stage whether detection probability needs to be taken into account, so that this can be borne in mind when designing the survey.

8 Outlook

Broader application

Since commercial cultivation of GMPs began in the mid-1990s, the global area on which they are grown has steadily increased. In 2016 GM crops covered 185 million hectares of land – an area more than five times the size of Germany. More than 97% of the global cultivation area is used for soya beans, maize, cotton and oilseed rape. As in the past, the predominant genetically engineered properties of GMPs are resistance to herbicides and pests. Around 80% of the GMPs cultivated in the laboratory or commercially have these properties (Tsatsakis et al. 2017). This currently one-sided focus of commercial green genetic engineering could become broader in the coming years. Current research and release trials with newly developed GMPs indicate that the engineered properties will become more diverse.

Adaptation to special locational conditions – for example, in the form of tolerance of drought, cold or heat – is a desired objective. GM soya for temperate climates, GM lupins and other GM legumes are candidates for future sources of protein. Stress-tolerant GMPs are of particular interest in connection with cultivation in developing and newly industrialising countries, where GMP crops are at present rarely grown.

Altered constituents and improved growth properties are also playing an increasingly important part in the development of GMPs. An example of this is a new GM maize line containing elevated levels of vitamin C, folic acid and provitamin A (ß-carotene). Also important for the European market are the release trials of GM potatoes that are resistant to blight. They contain resistance genes from wild potatoes.

Plants that serve as sources of energy are becoming increasingly important too. Plant species such as maize, poplar, sugar cane and millet (sorghum) are currently being genetically engineered to improve their suitability for bioenergy use and are being tested in release trials.

Advanced methods

Many new methods of genetic engineering have been developed in the last 10 years. Some of them will again open up radically new possibilities in plant breeding, because they are able to intervene in the DNA structure or in the regulation of gene expression with far greater precision than classical genetic engineering techniques. One of these new methods is CRISPR/Cas, which enables specific DNA sequences in a cell's genome to be added, altered or removed. The DNA is "revised", so to speak, which is why the technique is referred to as "genome editing".

At molecular level, plants modified using these new breeding methods differ very little from classically bred plants or natural genetic variants. It is likely that current detection and screening methods used to identify the presence of GMPs in the field, in agricultural production or in processed goods will soon be unable to recognise this new generation of GMPs.

Consequences for GM monitoring

New plant varieties are relevant to a GM monitoring programme if they are classed as GMPs in the eyes of the law. The genetic differences between plants bred using the new methods and natural varieties will often be smaller than the differences between currently approved GMPs and their natural relatives. The breeding process may not involve incorporating any DNA from foreign organisms, or it may be only the regulation of the genes that is altered. Policy-makers are being urged to define GMPs more precisely. The European Court of Justice ruled in July 2018 that the so-called new mutagenesis techniques fall under the EU GMO legislation[16]. Practical implications of this ruling are still discussed in the EU.

According to the result of the above-mentioned discussions, GMP monitoring programmes may need to be adapted. Provided that reliable molecular methods

[16] https://curia.europa.eu/jcms/upload/docs/application/pdf/2018-07/cp180111en.pdf

of detecting the target GMPs exist, the strategies and procedures outlined in this report can be applied with any necessary adjustment. The procedures that have been described are generally applicable. It is important to bear in mind the basic rule that for each GMP not only specific detection methods must exist but preliminary work must also be carried out in order to obtain information on possible origins, the flow of goods, relevant goods, exposure pathways and release locations. The data collection strategy must also be adapted to each GMP.

As the number of available GMPs increases, GM monitoring programmes may therefore become more demanding and more complex. This means that setting priorities when selecting the target GMPs for the monitoring programme will become all the more important. These priorities will in any case be heavily influenced by which GMPs pose the greatest risk to humans and the environment.

9 References

Bailleul D., Ollier, S., Huet, S., Gardarin, A., Lecomte, J. 2012. Seed spillage from grain trailers on road verges during oilseed rape harvest: an experimental survey. PloS ONE: https://doi.org/10.1371/journal.pone.0032752.

Bauer-Panskus A., Breckling, B., Hamberger, S., Schumm, M., Then, C. 2013: Cultivation-independent establishment of genetically engineered plants in natural populations: current evidence and implications for EU regulation. Environmental Sciences Europe 25. doi**:** 10.1186/2190-4715-25-34.

Bauer-Panskus A., Hamberger, S., Then, C. 2015 : Die unkontrollierte Ausbreitung gentechnisch veränderter Organismen – eine Übersicht über aktuelle Fälle und neue Risiken durch die Synthetische Biologie. Testbiotech, Munich. 40 pp.

BLW 2017: Agrarbericht. Bundesamt für Landwirtschaft BLW, Bern, 460 pp. https://agrarbericht.ch/de/service/dokumentation/publikationen. Downloaded on 8.3.2018.

Brown J. A., Salehi M, M., Moradi, M., Panahbehagh, B., Smith, D. R. 2013: Adaptive survey designs for sampling rare and clustered populations. Mathematics and Computers in Simulation 93: 108-116.

Busi, R., Powles, S. B. 2016: Transgenic glyphosate-resistant canola (Brassica napus) can persist outside agricultural fields in Australia. Agriculture, Ecosystems & Environment, 220: 28-34.

Chen G., Kéry, M., Plattner, M., Ma, K., Gardner, B. 2013: Imperfect detection is the rule rather than the exception in plant distribution studies. Journal of Ecology, 101(1): 183-191.

Courtier-Orgogozo V., Morizot, B., Boëte, Ch. 2017: Agricultural pest control with CRISPR- based gene drive: time for public debate. EMBO reports, May 16, 2017, embor.embopress.org. doi: 10.15252/embr.201744205.

Demeke T., Perry, D. J., Scowcroft, W. R. 2006: Adventitious presence of GMOs: Scientific overview for Canadian grains. Canadian Journal of Plant Science 86(1): 1-23, 10.4141/P05-114.

Devos Y., Hails, R.S., Messéan, A., Perry, J.N., Squire, G.R. 2012: Feral genetically modified herbicide tolerant oilseed rape from seed import spills: are concerns scientifically justified? Transgenic research 21: 1–21.

Dyer G.A., Serratos-Hernández, J.A., Perales, H.R., Gepts, P., Piñeyro-Nelson, A., Chávez, A., Salinas-Arreortua, N., Yúnez-Naude, A., Taylor, J.E., Alvarez-Buylla, E.R. 2009. Dispersal of Transgenes through Maize Seed Systems in Mexico. PLoS ONE: https://doi.org/10.1371/journal.pone.0005734.

Edwards T. C., Cutler, D. R., Zimmermann, N. E., Geiser, L., Alegria, J. 2005. Model-based stratifications for enhancing the detection of rare ecological events. Ecology 86: 1081-1090.

EU 2004: Question and Answers on the regulation of GMOs in the EU. MEMO/04/85, 22 pp. Download: http://europa.eu/rapid/press-release_MEMO-04-85_en.htm.

Greene, S.L.; Kesoju, S.R.; Martin, R.C.; Kramer, M., 2015. Occurrence of Transgenic Feral Alfalfa (Medicago sativa subsp. sativa L.) in Alfalfa Seed Production Areas in the United States. PLoS ONE: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0143296.

Guan Z.-J., Zhang, P.-F., Wie, W., Mi, X.C., Kang, D.-M., Liu, B. 2015: Performance of hybrid progeny formed between genetically modified herbicide-tolerant soybean and its wild ancestor. Annals of Botany 7: 121–128.

Guisan A., Broennimann, O., Engler, R., Vust, M., Yoccoz, N. G., Lehmann, A., Zimmermann, N. E. 2006: Using niche-based models to improve the sampling of rare species. Conservation Biology 20: 501-511.

Guisan, A., Tingley, R., Baumgartner, J. B., Naujokaitis-Lewis, I., Sutcliffe, P. R., Tulloch, A. I. T., Buckley, Y. M. 2013: Predicting species distributions for conservation decisions. Ecology Letters 16: 1424-1435.

Innovabridge 2016: Analyse von Raps- und Rübsensamen enthaltendem Vogelfutter im Detailhandel. Unpublizierter Bericht im Auftrag des Bundesamtes für Umwelt BAFU. 8 pp. (unpubl. report by FOEN, German only)

Innovabridge 2017: Analysis of (GE) Birdfeed and Sampling of Hotspots of (GE) Oilseed Rape. Unpublizierter Bericht im Auftrag des Bundesamtes für Umwelt BAFU. 17 pp. (unpubl. report by FOEN)

Kalinina O., Zellera, S.L., Schmid, B. 2015: Persistence of seeds, seedlings and plants, performance of transgenic wheat in weed communities in the field and effects on fallow weed diversity. Perspectives in Plant Ecology, Evolution and Systematics 17: 421-433.

Katsuta K., Matsuo, K., Yoshimura, Y., Ohsawa, R. 2015: Long-term monitoring of feral genetically modified herbicide-tolerant Brassica napus populations around unloading Japanese ports. Breeding Science 65: 265-275.

Kéry M., Royle, J. A. 2016: Applied Hierarchical Modeling in Ecology: Analysis of distribution, abundance and species richness in R and BUGS: Volume 1: Prelude and Static Models. Academic Press. 808 pp.

KLBS 2015: GVO-Überwachungs- und Monitoringaktivitäten 2014 in der Schweiz. Unpublizierter Bericht im Auftrag des Bundesamtes für Umwelt BAFU. 24 pp. (unpubl. report by FOEN, German only)

KLBS 2017: Methodenvergleich zwischen real-time PCR und ELISA-Schnelltest. Unpublizierter Bericht im Auftrag des Bundesamtes für Umwelt BAFU. 10 pp (unpubl. report by FOEN, German only)

Knispel A.L., McLachlan, S.M., Van Acker, R.C., Friesen, L.F. 2008: Gene flow and multiple herbicide resistance in escaped canola populations. Weed Science, 56 (1): 72-80.

Knispel A.L., McLachlan S.M. 2010: Landscape-scale distribution and persistence of genetically modified oilseed rape (Brassica napus) in Manitoba, Canada. Environmental Science and Pollution Research, 17(1): 13-25.

Lang A., Oehen, B., Ross, J-H., Bieri, K., Steinbrich, A. 2015: Potential exposure of butterflies in protected habitats by *Bt* maize cultivation: A case study in Switzerland. Biological Conservation 192: 369-377.

Lee B., Kim C.G., Park J.Y., Park K.W., Kim H.J., Yi H., Jeong S.C., Yoon W.K., Kim H.M. 2009: Monitoring the occurrence of genetically modified soybean and maize in cultivated felds and along the transportation routes of the Incheon Port in South Korea. Food Control, 20(3): 250-254.

MacKenzie D. I., Nichols, J. D., Lachman, G. B., Droege, S., Andrew Royle, J., Langtimm, C. A. 2002: Estimating site occupancy rates when detection probabilities are less than one. Ecology, 83(8): 2248-2255.

Menzel G.; Lünsmann, I., Middelhoff, U., Breckling, B., Schmidt, G., Tillmann, J., Schröder, W., Filser, J., Reuter, H. 2005: Gentechnisch veränderte Pflanzen und Schutzgebiete – Wirksamkeit von Abstandsregelungen. Naturschutz und Biologische Vielfalt 10: 1-164.

Mercer K.L., Andow, D.A., Wyse, D.L., Shaw, R.G. 2007: Stress and domestication traits increase the relative fitness of crop–wild hybrids in sunflower. Ecological Letters 10, 383–393. http://dx.doi.org/10.1111/j.1461-0248.2007.01029.x.

Mercer K.L., Emry, D.J., Snow, A.A., Kost, M.A., Pace, B.A., Alexander, H.M. 2014: Fitness of crop-wild hybrid sunflower under competitive conditions: implications for crop-to-wild introgression. PLoS ONE: https://doi.org/10.1371/journal.pone.0109001.

Nicolia A., Manzo, A., Veronesi, F., Rosellini, D. 2014: An overview of the last 10 years of genetically engineered crop safety research. Critical Reviews in Biotechnology 34: 1, 77-88, DOI: 10.3109/07388551.2013.823595.

Nishizawa T., Nakajiama, N., Aono, M., Tamaoki, M., Kubbo, A., Saji, H. 2009. Monitoring the occurrence of genetically modified oilseed rape growing along a Japanese roadside: 3-year observations. Environmental Biosafety Research 8.01: 33-44.

Pascher K. 2016: Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environmental Sciences Europe 28: 30. doi: 10.1186/s12302-016-0098-1.

Pascher K., Hainz-Renetzeder C., Gollmann G. and Schneeweiss G.M. 2017: Spillage of Viable Seeds of Oilseed Rape along Transportation Routes: Ecological Risk Assessment and Perspectives on Management Efforts. Frontiers in Ecology and Evolution 5:104. doi: 10.3389/fevo.2017.00104

Piñeyro-Nelson A., Van Heerwaarden, J., Perales, H.R., Serratos-Hernández, J.A., Rangel, A., Hufford, M.B., Gepts, B., Garay-Arroyo, A., Rivera-Bustamante, R., Álvarez-Buylla, E.R. 2009. Transgenes in Mexican maize: molecular evidence and methodological considerations for GMO detection in landrace populations. Molecular Ecology, 18 (4): 750-761.

Price, B. and Cotter, J. 2014: The GM Contamination Register: a review of recorded contamination incidents associated with genetically modified organisms (GMOs), 1997–2013. International Journal of Food Contamination, 1(1), 1-13.

Putnam D.H., Woodward, T., Reisen, P., Orloff, S. 2016: Coexistence and market assurance for production of non-genetically engineered alfalfa hay and forage in a biotech era. Crop Forage Turfgrass Manag. 2016, 2.

Royle J. A., Dorazio, R. M. 2008. Hierarchical modeling and inference in ecology: the analysis of data from populations, metapopulations and communities. Academic Press. 464 pp.

Saji H., Nakajima N., Aono M., Tamaoki M., Kubo A., Wakiyama S., Hatase Y., Nagatsu M. 2005: Monitoring the escape of transgenic oilseed rape around Japanese ports and roadsides. Environmental Biosafety Research, 4(4): 217-222.

Schafer M.G., Ross, A.A., Londo, J.P., Burdick, C.A., Lee, E.H., Travers S.E. Van de Water, P.K., Sagers, C.L. 2011: The Establishment of Genetically Engineered Canola Populations in the U.S. PLoS ONE: https://doi.org/10.1371/journal.pone.0025736.

Schönenberger N., D'Andrea, L. 2012: Surveying the occurrence of subspontaneous glyphosate-tolerant genetically engineered Brassica napus L. (Brassicaceae) along Swiss railways. Environmental Sciences Europe, 24 (1): 1-8. http://link.springer.com/article/10.1186/2190-4715-24-23.

Schulze J., Frauenknecht, T., Brodmann, P., Bagutti, C. 2014: Unexpected Diversity of Feral Genetically Modified Oilseed Rape (Brassica napus L.) Despite a Cultivation and Import Ban in Switzerland. PLoS ONE

Schulze J., Brodmann, P., Oehen, B., Bagutti, C. 2015: Low level impurities in imported wheat are a likely source of feral transgenic oilseed rape (Brassica napus L.) in Switzerland. Environmental Science and Pollution Research 22 (21): 16936–16942.

Snow A. A. 2012: Illegal gene flow from transgenic creeping bentgrass: the saga continues. Molecular Ecology 21: 4663–4664.

Swiss Seed 2016: Qualitätssicherungssystem von Swiss-Seed zur Verhinderung von unbeabsichtigten GVO-Verunreinigungen bei der Einfuhr von Saatgut. Unpubliziertes Dokument. 3 pp. (unpubl. document, German only)

Thompson S. K., Seber, G. A. 1996: Adaptive sampling. Wiley.

Tsatsakis A.M., Nawaz, M.A., Kouretas, D., Baliase, G., Savolainen, K., Tutelyan, V.A., Golokhvast, K.S., Lee, J.D., Yang, S.H., Chung, G. 2017: Environmental impacts of genetically modified plants: A review. Environmental Research 156: 818–833.

Van Denderen P.D., Tamis, W.L.M., van Valkenburg, J.L.C.H. 2010: Risico's van introductie van exotische plantensoorten, in het bijzonder uit het geslacht Ambrosia, via import van zaden voor met name veevoer en vogelvoer. Gorteria 34 (3): 65-85.

VDI 2011: Monitoring der Wirkungen von gentechnisch veränderten Organismen (GVO) - Floristische Kartierung von gentechnisch veränderten Pflanzen (GVP), ihren Kreuzungspartnern und Kreuzungsprodukten. Verein Deutscher Ingenieure VDI, Richtlinie 4330 Blatt 10: 2011-10. www.beuth.de.

Watrud L.S., Lee, E.H., Fairbrother, A., Burdick, C., Reichman, J.R., Bollman, M., Storm, M., King, G.J., Van de Water, P.K. 2004: Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. Proceedings of the National Academy of Sciences 101 (40): 14533-14538.

Wedlich K. V., Franzaring, J., Fangmeier, A. 2016: Entwicklung und Erprobung eines Konzepts für ein Monitoring von für den Import zugelassenem transgenem Raps nach Richtlinie 2001/18/EG. BfN-Skripten 430, Bundesamt für Naturschutz, Bonn - Bad Godesberg. 102 pp.

Yoshimura Y., Beckie, H.J., Matsuo, K. 2006: Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. Environmental Biosafety Research 5 (2): 67-75.

Zapiola M. L., Mallory-Smith, C.A. 2012: Crossing the divide: gene flow produces intergeneric hybrid in feral transgenic creeping bentgrass population. Molecular Ecology 21: 4672-4680.

10 Annex

Annex 1: SOP for the sampling of rapeseed for laboratory analysis in Switzerland (see also link**)**

SOP for the sampling of rapeseed for laboratory analyses

Bern, Switzerland, July 2018

In order to ensure the comparability of the results of different rapeseed samplings as part of the Swiss nationwide GM rapeseed monitoring programme, the sampling procedure must be standardised. The instructions contained herein include a detailed work protocol as well as a survey form for rapeseed sampling.

Timing of sampling

Seeds of rapeseed may germinate during the entire growing season and sampling can be undertaken from spring through to autumn. However, experience has shown that the periods of **late April to early June** and **late September to late October** are suitable for sampling as many plants die off during periods of prolonged drought at the height of summer or following seed production.

Material per sample

- **1 preferably fresh young leaf** (approx. 5 x 5 cm, min. 2.5 x 2.5 cm) per rapeseed plant[17]
- If plants are very small or at seedling stage, take the entire plant (without any soil)
- If only seed pods are available, seed pods can be sampled instead of leaves

If there is any uncertainty as to the plant species, then stems, flowers and seed pods should be collected in addition to leaves in order to facilitate later species identification.

Packaging / labelling of samples and completion of survey form

- Package samples in **sealable plastic bags (Minigrip)**
- Write the **sample number** clearly onto each bag. (Recommended practice is to write onto the plastic bag prior to sampling, using a waterproof marker.)
- **Enter** sample number, number of plants in the sample, estimate of the total number of all plants at the location (in the case of random sampling) as well as the location's geographic coordinates **onto the survey form[18].** Instead of establishing locations' coordinates, locations may also be marked on a map, together with the sample number.

[17] For notes on the identification of rapeseed please see Appendix 1

[18] See Appendix 2

Field sampling procedure

- **Location with rapeseed plants at a density of ≤ 30 plants / 4 m² (low density)**:
  - **All plants are sampled.**
  - Plants may be combined into **aggregate samples** of up to 10 leaf samples per plastic bag (**one** leaf from each plant).

As a general rule, plants growing within an area of 10 x 10 m may be combined into an aggregate sample. Where plants are growing at greater distances from each other, they must be treated as separate samples (cf. aggregate samples 1, 2 and 3; **Fig. 1**)

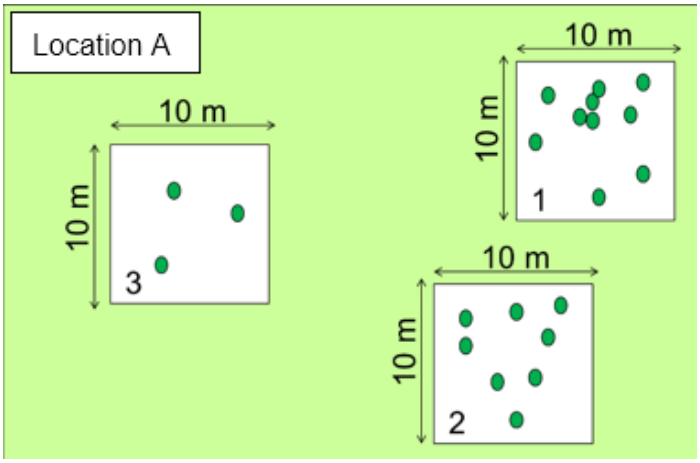
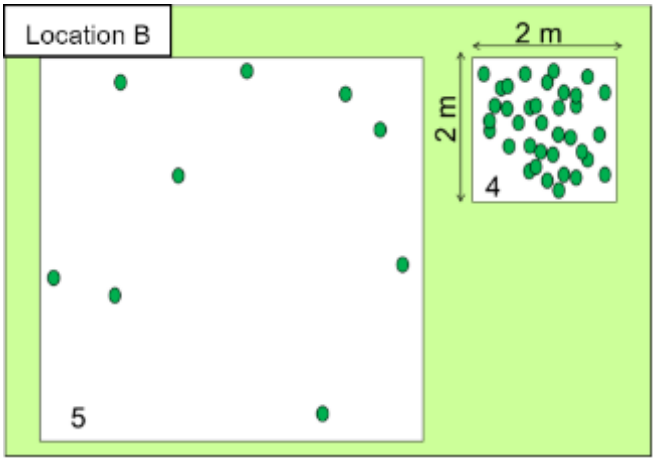


Fig. 1: Plants growing within an area of 10 x 10 m may be combined into an aggregate sample. Where plants are growing at greater distances from each other, they must be treated as separate samples (cf. aggregate samples 1, 2 and 3)

- **Location with rapeseed plants at a density of > 30 plants / 4 m² (high density)**:
  - The total number of plants is estimated and recorded in the survey form.
  - A **random sample comprising 20% of all plants present** is taken in the form of aggregate samples, with a minimum of 10 plants (cf. sample 4, **Fig. 2**).

There is a smooth transition between locations with high and low densities respectively. Where rapeseed plants are found at low densities in the direct vicinity of a location with a high density of plants, the former are similarly recorded as aggregate samples (cf. sample 5, **Fig. 2**).

Fig. 2: Where rapeseed is growing at a density of > 30 plants / 4 m^2, the total number of plants is estimated and recorded in the survey form, and a random sample comprising 20% of all plants present is taken in the form of aggregate samples, with a minimum of 10 plants (cf. sample 4: a total of 40 plants is present, 10 plants are sampled). Where additional rapeseed plants occur nearby at a low density, these are similarly recorded as aggregate samples (cf. sample 5).



RATIONALE: Due to the fact that dense clusters of plants are often derived from the same mother plant, in many cases they are found to be genetically similar. Therefore, random sampling is a trade-off between time and financial resources on the one hand and a complete knowledge of all genotypes at the location on the other.

- ***Special case: Seed pod sampling for GM rapeseed outcrossing analysis***

If **GM rapeseed had previously been found at a particular location**, seed material can be used to detect outcrossing of GM rapeseed into non-GM rapeseed or related species (e.g. charlock, brown mustard). Seed pod samples are collected to this end. The processing of seed pod samples is more complex than that of leaf samples. Therefore, the sampling of seed pods would appear to be expedient only at locations known to host GM plants.

  - **A sample of 5 pods per plant** (or all seed pods where there are fewer than 5) is taken and stored **in a separate plastic bag**. For each pod sample, a **leaf sample** is also collected **from the mother plant** to allow for an analysis of the mother plant's sample in case the seed samples test GM positive.

- ***Access to private properties***

Where samples must be taken on private land (e.g. company premises), it is mandatory that prior notice be given to the Canton in question and that permission be sought from the landowner.

Transport and storage

- **Transport**:
  - Samples are **stored in plastic bags**.
  - If temperatures are very high or in cases where samples can only be refrigerated after more than approx. 4 hours, samples should immediately be stored **in a cool bag** containing cooling elements (not directly on the cooling elements to prevent samples from freezing).

- **Storage**
  - **Prior to analysis**, samples may be **stored for appr. 5-7 days in a fridge**.
  - If samples cannot be processed within this period (DNA extraction, Quickstix test) they must be frozen. While technically it is not a problem to process frozen and defrosted plant material, it is easier to work with fresh material.
  - **Clearly labelled reference samples must be kept and frozen of all analysed samples** so as to allow for re-analysis of samples. Where aggregate samples are frozen, care must be taken to ensure that the individual leaves do not break in order to allow for definitive quantification of GM rapeseed in aggregate samples.

Shipping of samples

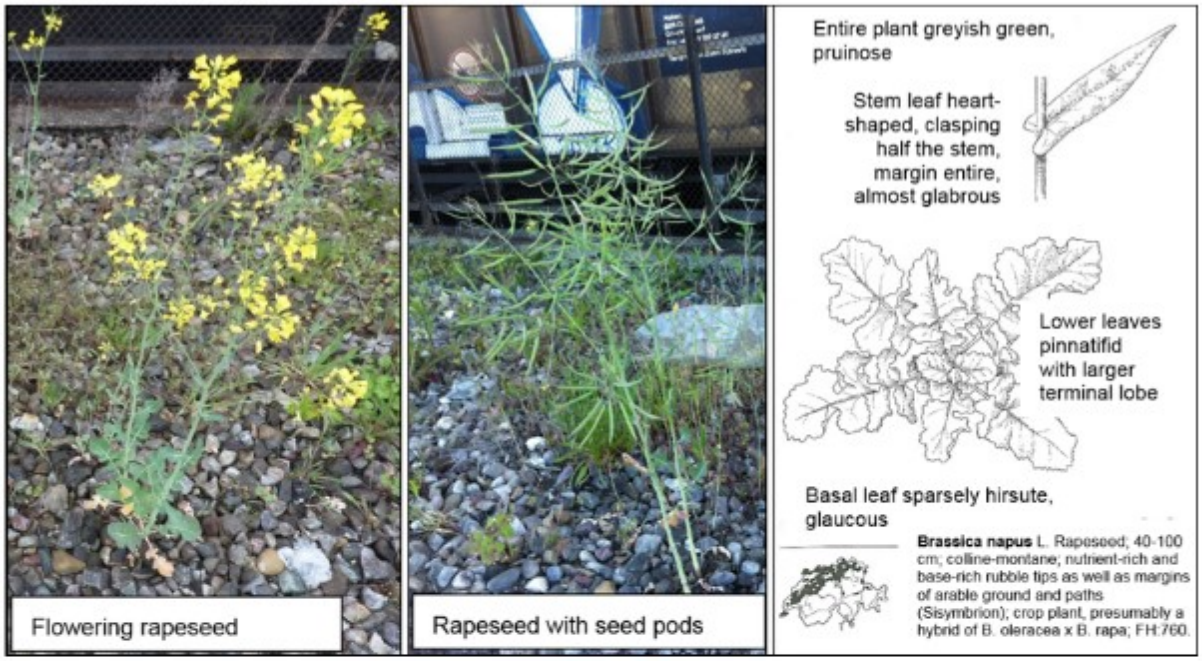
- Fresh or frozen samples may be **mailed in insulated packaging (styrofoam box, cool bag) including cooling elements** as long as the samples reach the recipient address within 24 hours (do not place samples directly onto cooling elements to prevent them from freezing).
- Sample bags must be sealed tightly and inspected for rips or punctures to prevent any liquids from escaping.
- In the case of frozen aggregate samples great care must be taken to absolutely ensure that none of the individual sample leaves get broken.

Appendix 1

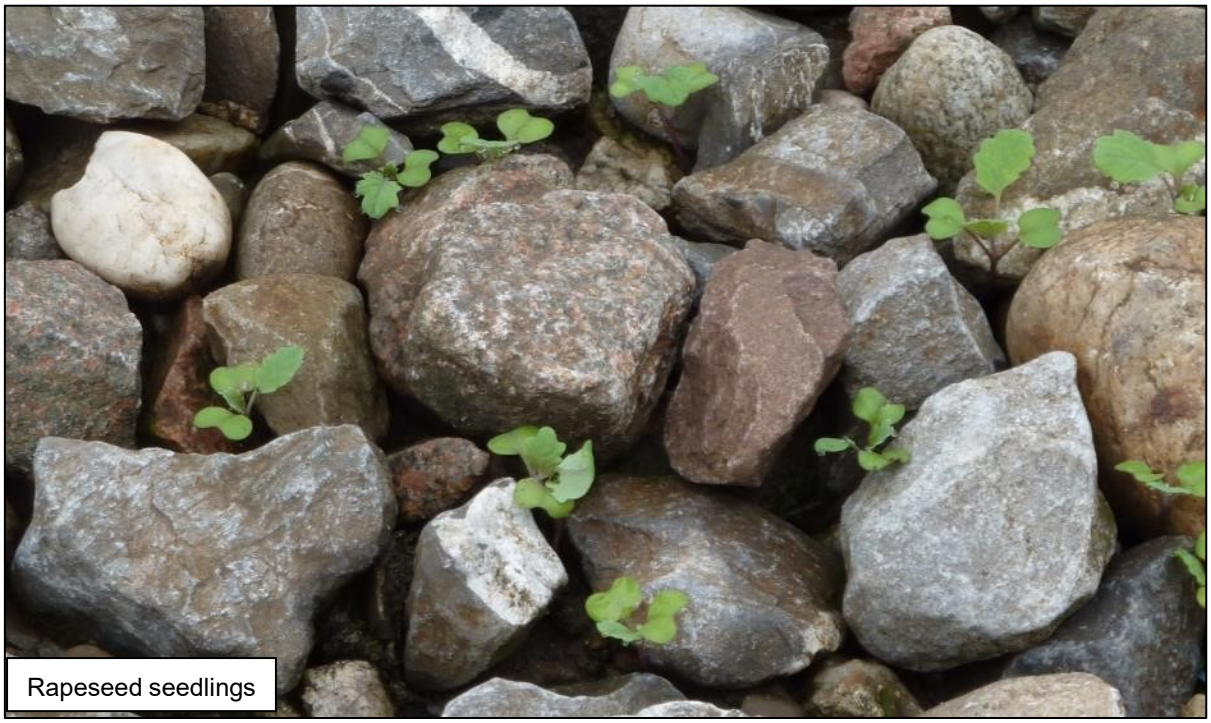
Identification of rapeseed

It is possible to identify rapeseed even without special botanical knowledge. However, rapeseed may appear in very different growth forms, and especially in wild populations it often occurs merely in dwarfed form without its typical basal leaves (**Fig. 3**). Greater experience is needed for the positive identification of small rapeseed plants without inflorescences as they may be confused with similar looking relatives (e.g. charlock or white mustard; **Fig. 4**). However, individual wrongly identified plants cause only minor distortions to the total sample count and calculations as to the proportion of GM rapeseed and therefore are not generally a problem. Where plant species identification is uncertain, a sample containing an intact leaf, stem, flowers and seed pods should be taken if possible. This allows for retrospective identification of samples by experienced botanists.

Fig. 3: Various growth forms of rapeseed, *B. napus* and diagram of vegetative plant (after Eggenberg/Möhl: “Flora Vegetativa”, 2nd edition, Haupt Verlag)



Rapeseed with flowers and seed pods



Rapeseed seedlings

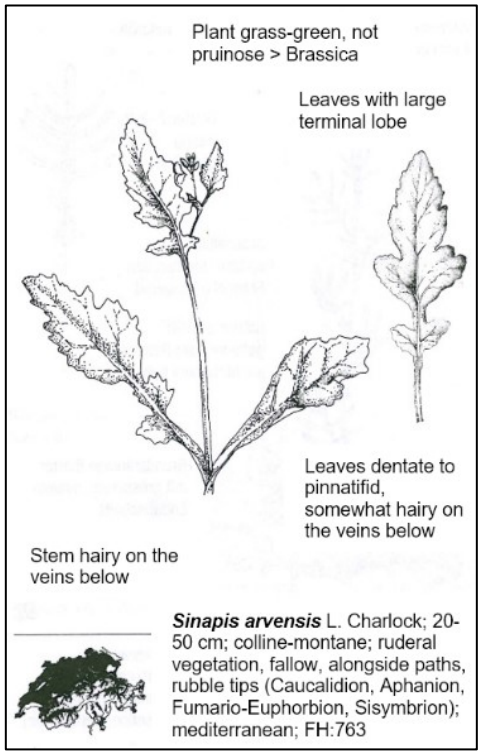
Medium-sized rapeseed without flower and few seed pods

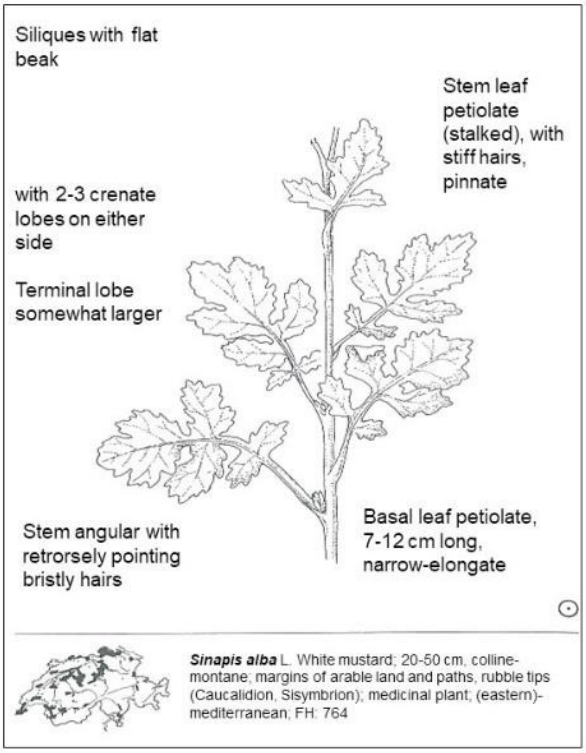
Medium-sized rapeseed and seedlings

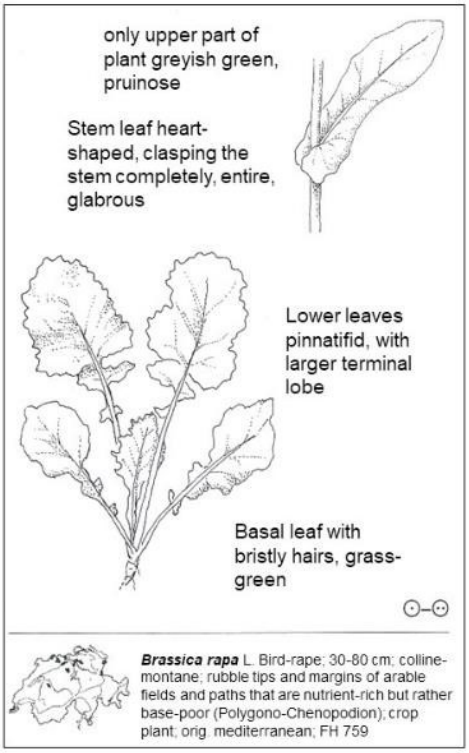
Medium-sized rapeseed and young seedlings

Fig. 4: Selection of plant species that are easily confused with rapeseed (photograph and diagram of Charlock, *S. arvensis,* diagrams of White mustard, *S. alba* and Bird-rape *B. rapa;* diagrams after Eggenberg/Möhl: "Flora Vegetativa", 2nd edition, Haupt Verlag)

Charlock (*Sinapis arvensis*)







Appendix 2

Survey form for sampling rapeseed

(separate sheet to be completed for each sampling location)

Sampling location:	
Sampler:	**Date:**
Notes:	

Sample number	Number of plants in sample (1 to 10)	For random samples: Estimate of total number of plants / Notes	Geographic coordinates (or GPS waypoint). Alternatively, marker on map.