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Federal Department of the Environment,
Transport, Energy and Communications DETEC

Federal Office for the Environment FOEN
Division Soil and Biotechnology

SOP for the sampling of rapeseed for laboratory analyses

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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 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[1]
- 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 data collection

- Package samples in **sealable plastic bags (Minigrip)** and write the **sample name** clearly onto each bag.
- Data collection in the field is done with the GMO application (www.gmo-monitoring.ch) on a smartphone or tablet. In order for the data to be recorded online, an account for the application must first be requested from the FOEN (e-mail to contact.releases@bafu.admin.ch). All data can be entered directly in the field in the application. The sample names are unique and are automatically generated by the system as soon as sampling is started. The sample names should then also be written on the plastic bags.

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

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**)

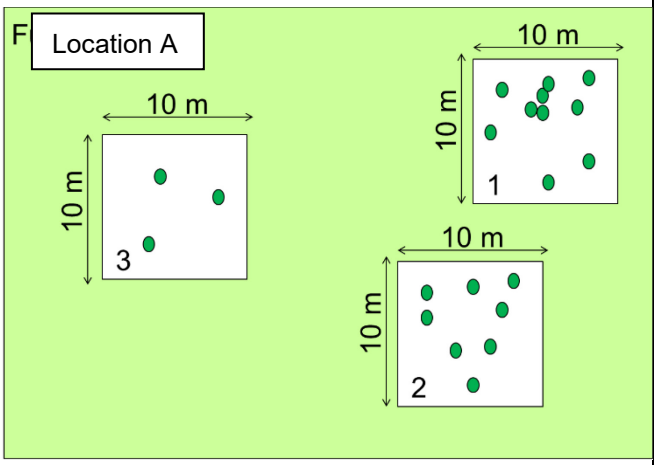


Fig. 1: Plants growing within an area of 10 x 10 m may be combined into an aggregate sample. Where plants are growing at greater distances from each other, they must be treated as separate samples (cf. aggregate samples 1, 2 and 3)

- **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**).

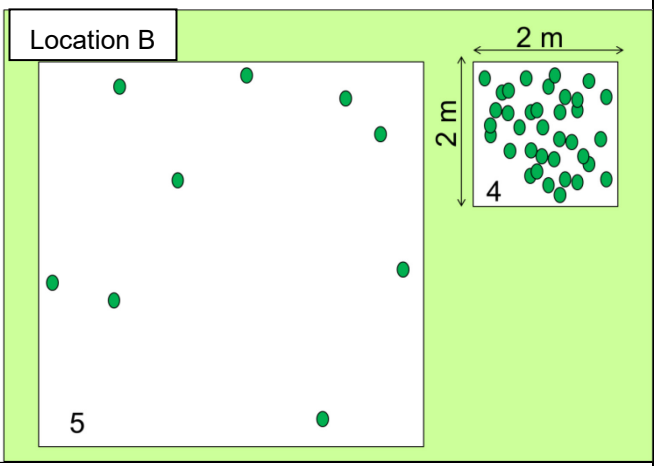


Fig. 2: Where rapeseed is growing at a density of > 30 plants / 4 m², the total number of plants is estimated and recorded in the survey form, and a random sample comprising 20% of all plants present is taken in the form of aggregate samples, with a minimum of 10 plants (cf. sample 4: a total of 40 plants 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

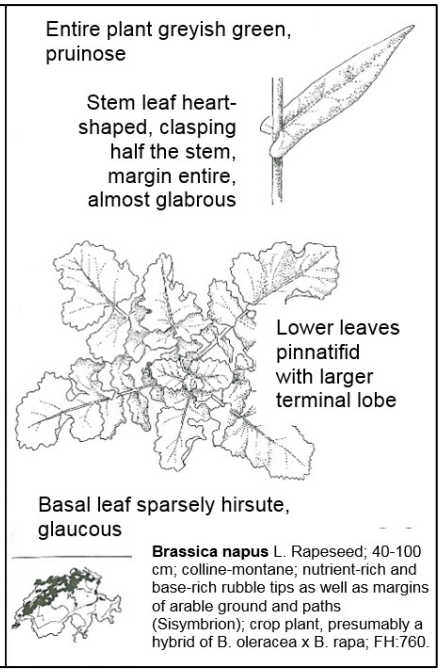
Identification of rapeseed

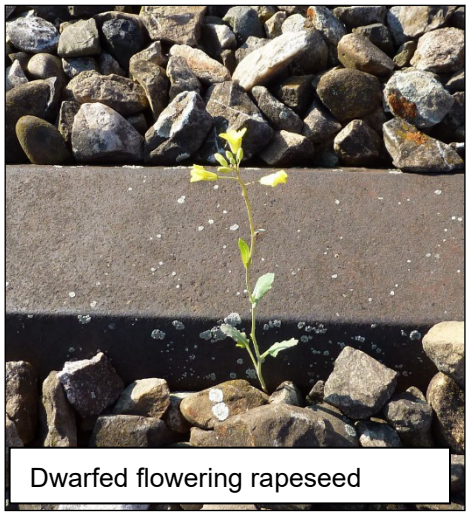
It is possible to identify rapeseed even without special botanical knowledge. However, rapeseed may appear in very different growth forms, and especially in wild populations it often occurs merely in dwarfed form without its typical basal leaves (**Fig. 3**). Greater experience is needed for the positive 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)


Flowering rapeseed


Rapeseed with seed pods



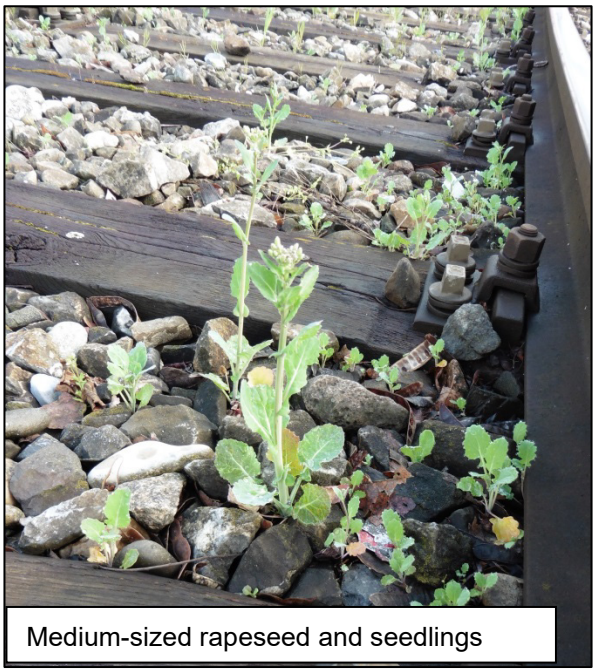

Dwarfed flowering rapeseed


Clustered young rapeseed plants

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)