

Which soils are comfortable for oilseed rape seeds (*Brassica napus*) to survive?

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ABSTRACT

The study should reveal the effects of soil texture and of seed characteristics of oilseed rape (*Brassica napus* L.) on seed survival in the soil by a burial experiment. Seeds of 58 conventional and new trait accessions (genetically modified and/or with altered seed ingredients) were buried for six months in sandy loam (SL), clay (C) or silty clay loam (SICL) in the seasons 2008/2009 and 2009/2010 in Germany. Between 84–93% (2008/2009) and 64–87% (2009/2010) of the conventional seeds survived in the soil, and so did 41–67% and 8–44% of the new trait seeds. Significantly more seeds were found in C and SICL compared to SL. Seed survival also significantly depended on the genotype, with high and low seed survival in both conventional and new trait accessions. Seed survival rates are probably linked with moisture and oxygen provided by different soils. As soils with good water storage conditions seem to have a higher probability for oilseed rape seeds from harvest losses to survive, other measures such as growing low dormancy varieties and proper soil tillage are especially relevant to avoid volunteers on these soils.

Keywords: dormancy; GMO; sand; persistence; soil texture

Oilseed rape (canola; *Brassica napus* L.; OSR) is a major oil crop in temperate regions of Europe. Huge seed losses during mechanical harvest of on average 4000 seeds/m², corresponding to a 100-fold of the regular sowing density, respectively, dropping onto the soil (Weber et al. 2009), can result in large and long-lasting soil seed banks. The seed survival of OSR in the soil is mainly caused by secondary dormancy that seeds acquire when they get buried by soil tillage under specific ambient conditions, such as water stress and darkness (Pekrun et al. 1997, Weber et al. 2010). The soil seedbank stands for a long-time source for volunteers with weedy effects in all crops. Furthermore, particular volunteers can spoil the quality of the harvest of any following OSR in the same area by seed admixtures. This issue can apply to genetically modified OSR, which, however, is not an option at the moment in Europe. Currently, the issue of volunteers is rather related to seed ingredients, for instance the pattern of fatty acids, which can be spoiled if seeds from another (volunteer) variety are mixed with the grown crop. Another new debate about OSR volunteers was raised in the European public along

with commercial cultivation of herbicide tolerant varieties from conventional breeding (Clearfield[®] system), which are tolerant to acetolactate synthase inhibitors. Thus, OSR volunteers remain still an issue of research, and measures for their control should be further developed.

It is well known that the size of the soil seed bank depends on the mode and time of post-harvest tillage (Pekrun et al. 1998, Gruber et al. 2010), and on the genotype (Momoh et al. 2002, Gruber et al. 2009). Both factors can theoretically be controlled by appropriate management practice. External factors, however, such as temperature or soil moisture, which are crucial factors for dormancy induction, cannot be influenced and result from interaction of current weather conditions with soil texture. A regression analysis from a survey of farmers' fields in Germany revealed cropping frequency and region (soil/climate) as most relevant factors determining the numbers of OSR volunteers (Gruber et al. 2012). A more specific definition of the soils that offer a higher risk for volunteers would help farmers to better plan their agronomic measures beforehand. There was no field trial for OSR until now where different

soils were tested for seed survival under the same environmental conditions which exclusively allows determining the effect of soil.

The study should give evidence (i) whether the size of the soil seed bank of OSR varies depending on soil texture; (ii) whether seed survival depends on seed characteristics; (iii) which soil characteristics could be responsible for differences in seed survival. The seed survival was tested in a burial experiment.

MATERIAL AND METHODS

The experiment took place at two fields (field 1, field 2) at the research station Ihinger Hof of the University of Hohenheim/Germany (48°44'N, 8°56'E, 478 m a.s.l., Ø 8.1°C, Ø 693 mm) during the seasons 2008/2009 and 2009/2010. Three different soils were filled in excavated holes (1.0 × 1.1 m/hole) in the ground at both fields in a depth of 0–20 cm. The soils had contact to the *in situ* soil (silty clay loam) under the filled soil material, and were isolated from the natural ambient soil and among each other by wooden planks to avoid lateral flow of soil material. The soils for seed burial (sandy loam, SL; clay, C; silty clay loam, SICL) were obtained from three farms in Southern Germany, one of them the original soil from the experimental site (field 1) Ihinger Hof, and differed in soil parameters such as texture, water content and nitrogen content (Table 1).

The experimental design for field 1 was a split-plot design with three replicates (blocks), where the three soils were used as main plots, and the OSR accessions as sub-plots. In field 2, the main plots (soils) were arranged in a Latin square design, and sub-plots (seed samples from different OSR accessions) were completely randomised in each main plot. Soils were brought to the experimental area after dry storage, crushed *in situ* by a hand rake and slightly re-compacted. The soils remained *in situ* during the seasons 2008/2009

and 2009/2010. Weeds were removed by hand. Seed samples from conventional OSR were derived from official German field trials (harvested 2008 and 2009) and were buried in field 1. Seed samples from accessions with altered seed ingredients ('new trait accessions') were obtained from breeders and were derived from harvest 2008; they were buried in field 2, together with the standard varieties Smart and Express (Table 2).

Seeds were placed in polyester bags (10 × 10 cm) with a mesh width of < 0.5 mm in a number of 500 seeds per treatment × replicate. All bags were buried in a depth of 10 cm and protected by mesh wire from predation by birds and mammals. Only seeds which fell dormant could survive under these conditions because germinated seeds were not able to emerge and perished in the bags.

To generate dry conditions which induce dormancy, the burial areas were protected from precipitation four weeks before and three weeks after burial, namely from 6th August 2008–25th September 2008, and 16th July–3rd September 2009, respectively. In field 1, the experimental area of 6 m² per replicate was kept dry under mobile rain shelters made of wooden slats and plastic foil. The rain shelters were used for the conventional accessions. The new trait accessions were buried in field 2 in an open-air greenhouse to comply with the regulations for GMOs. The outer structure of the greenhouse was a solid frame combined with a wire mesh, which could be covered or uncovered by plastic foil. After six months burial, seeds of both trials were exhumed at the beginning of the growing season in March 2009, or 2010, respectively. Seeds were counted and germinated at 20°C under light and a following treatment with alternating light and temperature conditions according to Weber et al. (2010). The seeds which germinated during this two-phase germination test were classified as survivors with full viability.

The statistical evaluation was performed by the procedure Glimmix with help of the statistical programme SAS 9.3 (SAS Institute Inc. Cary,

Table 1. Properties of three soils used for a burial experiment of oilseed rape seeds at Ihinger Hof/Germany

| Soil texture | Mineral particles (%) | | | C _t (% DM) | Soil moisture (weight %) ¹⁾ | | Mineral N ²⁾ (kg/ha) | Bulk density ²⁾ (g/cm ³) | pH ²⁾ |
|--------------|-----------------------|-------|-------|-----------------------|--|-------|---------------------------------|---|------------------|
| | sand | silt | clay | | 2008 | 2009 | | | |
| SICL | 1.31 | 69.70 | 28.99 | 0.64 | 6/8 | 11/10 | 40 | 1.45 | 7.2 |
| C | 21.14 | 35.09 | 43.77 | 3.77 | 17/17 | 16/18 | 237 | 0.87 | 7.0 |
| SL | 64.43 | 23.89 | 8.22 | 0.66 | 4/3 | 5/4 | 32 | 1.34 | 5.1 |

SICL – silty clay loam; C – clay; SL – sandy loam (USDA 2013). ¹⁾before burial in each year; moisture: field 1/field 2; ²⁾before burying 2008. C_t – total carbon; DM – dry matter

Table 2. Winter oilseed rape (*Brassica napus* L.) accessions used for seed burial experiments 2008/2009 and 2009/2010 at the experimental station Ihinger Hof/Germany

| | 2008/2009 | 2009/2010 |
|-----------------------|---|--|
| Field 1: conventional | Alkido, Aragon, Baldur, Billy, Canti, Cindi, Cooper, Elektra, Expert, Express, Fangio, Favorit, Hycolor, Ladoga, Lilian, Lioness, Lorenz, Nemax, PR4631, Smart, Taurus, Tenno, Trabant, Zeppelin | Adriana, Baldur, Cindi, Cooper, Elektra, Express, Fangio, Favorit, Hammer, Komando, Ladoga, Lorenz, Nemax, PR46W15, PR46W31, Petrol, Smart, Taurus, Vision, Zeppelin |
| Field 2: new traits | Standards and isogenic lines: Drakkar ¹⁾ , Lisora ²⁾ , Smart, Express GM with altered ingredients: RedSin (Reduced Sinapin), Resvera (with Resveratrol) Conventional with altered ingredients: Thick ³⁾ , Thin, Holli 1 ⁴⁾ , Holli 2, Yellow 1 ³⁾ , Yellow 2, Yellow 3 ⁵⁾ , Black ^{3,5)} | |

¹⁾isogenic to RedSin; ²⁾isogenic to Resvera; ³⁾Black, Thick – high seed fibre; Yellow – low seed fibre (Gruber et al. 2012); ⁴⁾High Oleic, Low Linoleic; ⁵⁾only in 2009/2010

USA). For the evaluation of seed burial, data were processed using the logit transformation. The model for the Glimmix procedure was:

$$\text{survived seeds/total seeds} = \text{subplot} \times \text{replicate (soil)} + \text{soil} + \text{accession} + \text{soil} \times \text{accession (field 1)}$$

$$\text{survived seeds/total seeds} = \text{column} \times \text{replicate (soil)} + \text{soil} + \text{accession} + \text{soil} \times \text{accession (field 2)}$$

Where: residuals (both fields), and subplot \times replicate (field 1) or column \times replicate (field 2), respectively, were considered as random.

RESULTS AND DISCUSSION

All remaining, unearthed seeds appeared intact and had germinability of > 99% (data not shown). Soil seed survival ranged from 84–93% (2008/2009) and 64–87% (2009/2010) for the conventional accessions, and 41–67% and 8–44% for the new trait accessions (Figure 1). The survival rate was lower in the second year, which is probably a result of seed ageing for the new trait accessions (seeds derived from harvest 2008). Soil texture and accession most significantly affected seed survival (Table 3).

Interactions of soil \times accession were only found for conventional accessions in the first year: SL resulted in higher discrimination of the survival rates (data not shown). Significantly lower survival with approximately 10–80% less seeds surviving occurred in SL in both groups of accessions (Figure 1).

For the conventional accessions, there was genotypic variation in seed survival similar to the study of Gruber et al. (2004). Accessions with low fibre content seemed to have survived to a lesser extent than accessions with high fibre content,

though molecular-genetic studies did not show a link between fibre content and seed dormancy as a pre-condition for seed survival (Schatzki et al. 2013). The variation among the new trait accessions (Figure 2) can be also well explained by the known genotypic variation and disposition to dormancy that ranged between 1% (Yellow 1) and 59% (Thick; data not shown) and that significantly correlated with seed survival (Weber et al. 2011).

Moisture, temperature, nutrients and anoxia can be considered relevant soil characteristics involved in induction of and release from dormancy. Generally, under soil water potential and temperature adjusted to the same level seed survival seems similar in different soil types (Long et al. 2009).

The results from Lutman et al. (2003) correspond to ours, with higher seed survival of OSR in a silty clay than in a sandy loam; other results, however,

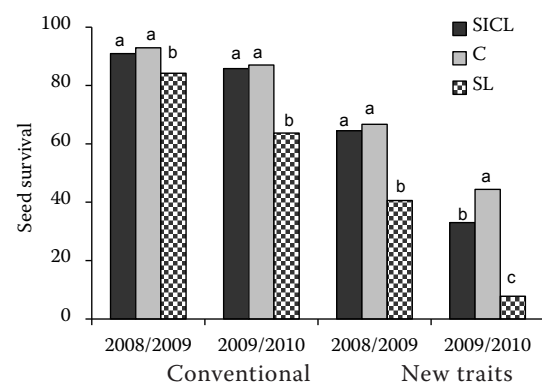


Figure 1. Survival (%) of oilseed rape seeds from conventional and new trait accessions after six months burial in silty clay loam (SICL), clay (C) and sandy loam (SL) at Ihinger Hof, Germany, in two seasons. Values with the same letters are not significantly different at $P < 0.05$; comparisons only within year \times genotype

Table 3. Variances for survival of oilseed rape seeds from conventional and new trait-accessions, after six months burial in different soils in two seasons at Ihinger Hof/Germany

| | Effect | | |
|-------------------------------|----------|-----------|------------------|
| | soil | accession | soil × accession |
| 2008/2009 conventional | | | |
| df | 2 | 23 | 46 |
| F-value | 14.56 | 14.94 | 1.74 |
| P > F-value | < 0.0001 | < 0.0001 | 0.0078 |
| 2009/2010 conventional | | | |
| df | 2 | 19 | 38 |
| F-value | 27.21 | 4.36 | 1.46 |
| P > F-value | < 0.0001 | < 0.0001 | 0.0673 |
| 2008/2009 new traits | | | |
| df | 2 | 11 | 22 |
| F-value | 9.81 | 9.92 | 1.30 |
| P > F-value | 0.0002 | < 0.0001 | 0.2055 |
| 2009/2010 new traits | | | |
| df | 2 | 13 | 26 |
| F-value | 19.30 | 12.47 | 1.15 |
| P > F-value | < 0.0001 | < 0.0001 | 0.3133 |

seem different (Pekrun et al. 1998). These differences can have been caused by the described variation in rainfall among the experimental sites of Pekrun et al. (1998). High soil water tension is a reason for low germination of OSR (Williams and Shaykewich 1971) and one of the pre-conditions for induction of seed dormancy (Pekrun et al. 1997, Weber et al. 2010). High soil water tension is determined by (i) water storage capacity of the soil (depending on the soil texture), and (ii) by precipitation. The water contents of the three soils in our experiment was actually different at the

time of seed burial (Table 1), but all soils provided similar dry conditions beyond the permanent wilting point (AG Boden 2005). All following rainfall was the same for both areas and soils, thus the results could only refer to the differences in soil.

Annual ryegrass (*Lolium rigidum*) seeds from a pot experiment had a greater number of viable and primarily dormant seeds surviving in a sandy loam compared to a clay soil (Narwal et al. 2008), but this grass species had a generally short survival time, and there was no further induction of dormancy. In contrast, OSR seeds survive 10 years and more (Lutman et al. 2003, D’Hertefeldt et al. 2008), and primary dormancy is usually zero or low in mature seeds (Gruber et al. 2004). Thus all surviving OSR seeds in the current study must have fallen dormant during seed burial. Consequently, if clay soils tend to provide conditions for non-dormant seeds to perish (Narwal et al. 2008), seeds with long-term dormancy or dormancy induction such as OSR could well survive.

Hypoxia is another factor which can induce dormancy (Benvenuti and Macchia 1995, Pekrun et al. 1997, Momoh et al. 2002). Sandy soils with greater pore volume and gas exchange facilitated emergence of *Datura stramonium*, whereas emergence was inhibited by clay soils (Benvenuti 2003). Also soil compaction which might be stronger in clay soils can impede germination of weed seeds, at least partly due to hypoxia (Pareja and Staniforth 1985) and to soil moisture (Terpstra 1995).

For OSR, we hypothesize the following scenario: phase 1 is dormancy induction within the first weeks after seed burial while the seeds are under dry conditions (Pekrun et al. 1998, Lutman et al. 2003, Gruber et al. 2010). More seeds fall dormant under dry conditions, in interaction with their genotypic disposition. In phase 2, at exposure of the areas to

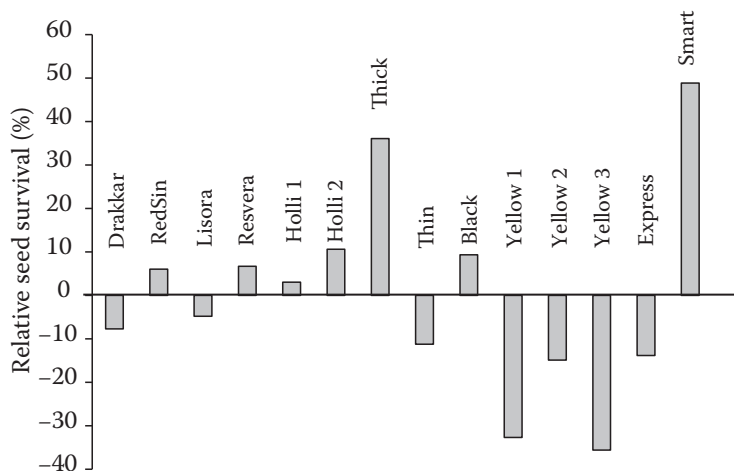


Figure 2. Relative seed survival rates (= deviation from variety mean across two seasons and three soils) of new trait oilseed rape accessions and standards, buried for six months at Ihinger Hof/Germany; means of two seasons and three soils

natural precipitation, a higher release from dormancy and (fatal) germination of rapeseeds buried in sandy soils occurred due to higher gas exchange rates, and maybe also because of higher temperature fluctuation due to the low heat capacity of air. Fluctuations in temperature are an effective way to break dormancy in OSR (Weber et al. 2010). A burial experiment with seeds unearthed at weekly intervals could give further evidence about the temporal progress of dormancy development and dormancy breaking in contrasting soils. Agronomic measures such as the choice of a low-dormancy variety and suitable tillage operations seem to be particularly important in fields with heavier soils.

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