Seed persistence of novel-trait oilseed rape (*Brassica napus* L.) genotypes in different soils

E.A. Weber, K. Frick, F. Stockmann, S. Gruber, W. Claupein
University of Hohenheim, Institute of Crop Science, Fruwirthstr. 23, D-70599 Stuttgart, Germany

Introduction

Modern oilseed rape (OSR; *Brassica napus* L.) cultivars still exhibit some wildflower characteristics such as low shattering resistance of mature pods and the ability of seeds to persist in the soil for a long time. Both seed rain at harvest and the capacity of the seeds to survive in the soil account for the occurrence of volunteers in following crops. OSR volunteers are then unwanted because they are weeds. If novel-trait or GM (genetically modified) genotypes and conventional cultivars are grown in the same crop rotation they might cause pollen-mediated gene flow and direct seed admixture. Seed persistence and occurrence of volunteers within the crop rotation can be somewhat controlled by adjusted soil tillage (Gruber et al. 2004a), preventing early seed burial and thus dormancy induction in dry soil layers without sunlight. Relying only on soil tillage alone to reduce OSR seed persistence may not be safe enough, however, as tillage can fail or need adjustment to other agronomical demands.

Seed persistence of GM and conventionally bred OSR is known to be strongly correlated with the genotypic predisposition to seed dormancy (Gruber et al. 2004b), but may also be affected by the attractiveness of the seeds to pest organisms or to seed aging. OSR cultivars can differ significantly in their predisposition to dormancy (Gruber et al. 2009), ranging from almost 0 % to nearly 100 % (Weber et al. 2010). Whether OSR cultivars with novel traits behave differently is not known to date. For example fatty acid composition is expected to influence dormancy and germination, as has been suggested by Walker et al. (2004). Soil texture could play another important role for seed persistence due to differing conditions such as temperature, aeration and water supply, conditions that maintain or break dormancy. Previous studies on this topic have given some indications but have not provided exact data on whether or to what extent soil texture affects seed persistence of oilseed rape. The aim of this study was to assess the impact of genotype and soil texture on seed persistence of GM and conventionally bred novel-trait OSR.

Material and methods

The experiment was carried out in 2008/2009 and 2009/2010 in an open-air S1 facility (isolated from the environment by a solid small mesh wire) at the experimental station Ihinger Hof (48° 44' N, 8° 56' E, 478 a.s.l., Ø 8.1°C, Ø 693 mm). It was arranged in a split-plot design with three replicates. Two GM genotypes (sinapine reduced: RedSin; resveratrol enriched: Resvol), their near-isogenic counterparts (Drakkar, Lisora), six (2008) and eight (2009) conventionally bred OSR cultivars with modified traits (seed coat quality: Yellow 1, Yellow 2, Yellow 3, Thin, Thick, Black; fatty acid composition: Holli 1 and Holli 2) and two reference cultivars with low (Express) and high (Smart) seed dormancy were investigated (Tab. 1).

Seeds (500 per replicate) were enclosed in polyester mesh bags and buried (in September 2008 and 2009) in plots with three different soils (silty clay: sC; clay loam: cL; loamy sand: lS; Tab. 2) at a depth of 10 cm for 6 months. Before seeds were buried, seed dormancy was determined according to the Hohenheim dormancy test described by Weber et al. (2010). As no freshly harvested seeds were available in the second year, seed lots from the first year were used once again.

Tab. 1: Scheme of oilseed rape genotypes and their respective traits investigated in the burial experiment 2008/2009 and 2009/2010. Traits: seed coat color[1)], NDF[2)], ADF[2)], ADL[2)] and major fatty acids[2)]. Ref. - reverence

G.type	Breed.mode	Traits						Comment	Harvest
		Colour	NDF	ADF	ADL	C 18:1	C 18:3		
RedSin	GM	-	-	-	-			red. sinapine	
Drakkar	Conv.	-	-	-	-			near-isogenic to Redsin	2008
Resvol	GM	-	-	-	-			resveratrol enriched	
Lisora	Conv.	-	-	-	-			near-isogenic to Resvol	2008
Yellow1	Conv.	4.4	14.6	10.3	3.4	-	-		2008
Yellow2	Conv.	2.0	-	10.5	3.4	-	-		2008
Yellow3	Conv.	4.4	16.6	11.4	3.1	-	-	only 2009	2008
Black	Conv.	9.0	-	17.6	10.4	-	-	only 2009	2008
Thin	Conv.	9.0	14.1	12.0	6.6	-	-		2008
Thick	Conv.	9.0	21.0	14.9	7.8	-	-		2008
Express (ref)	Conv.	-	-	-	-			low-dormancy	2008
Smart (ref)	Conv.	-	-	-	-			High dormancy	2008
Holli1	Conv.	-	-	-	-	79	2.8		2008
Holli2	Conv.	-	-	-	-	79	3.4		2008

1) Colour determined according to a rating scheme from 1-9 (1-yellow, 9-black)
2) NDF, ADF and ADL, C 18:1 and C 18:3 referring to dry weight

The soil was covered by a canopy 4 weeks before and 3 weeks after burial to prevent rainfall and induce dormancy. Seeds were exhumed at the start of the following vegetation periods in March. The number of intact seeds was counted and viability was assessed by a germination test including a dormancy breaking procedure. Data were analyzed using the procedure GLIMMIX of the statistical software package SAS 9.2 (SAS Institute Inc. Cary, NC, USA).

Tab. 2: Soil texture of the investigated soils in the burial experiment

Soil texture	Composition (%)			
	sand	silt	clay	C_t
Silty clay (sC)	1.32	74.10	28.99	0.63
Clay loam (cL)	21.14	37.40	43.77	3.60
Loamy sand (lS)	64.43	23.89	8.22	0.66

Results and discussion

Seed persistence was significantly influenced by genotype in both years and varied between 7 and 95 % in 2008/2009 (Fig. 1). Lowest seed persistence (6.9 %) was observed for the yellow coated cultivar "Yellow 1". Cultivar "Thick" with a high crude fiber content in the seed coat showed a high persistence rate, comparable to the reference for high dormancy "Smart" (92.2 % and 95.9 %, respectively). All other cultivars ranged between 40 % and 70 % seed persistence. Both GM genotypes showed comparable dormancy values of about 57 % and did not differ significantly from their near-isogenic counterparts.

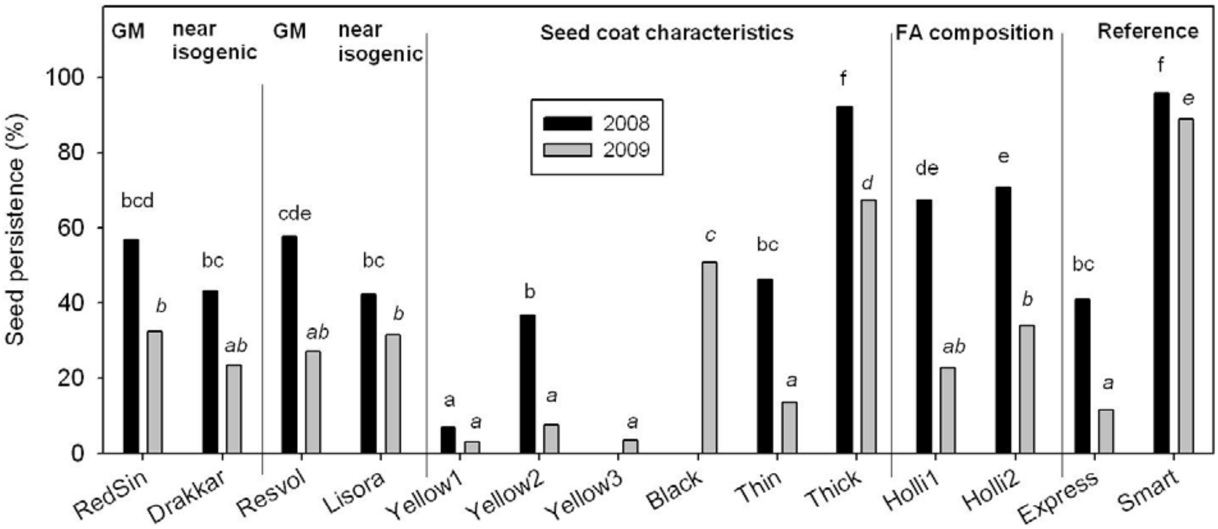

Fig. 1: Seed persistence as percentage of viable seeds after burial over 6 months (from September 2008 and 2009 till March 2009 and 2010, respectively) in 10 cm soil depth. Genotypes in the same season assigned the same letter are not significantly different (p<0.05).

In the season 2008/2009, the level of secondary dormancy correlated well with seed persistence with a R^2 of 0.79, indicating that the genotypic dormancy level might have overlapped a possible effect of variation in seed attractiveness or aging of the novel-trait genotypes on seed persistence (Fig. 2).

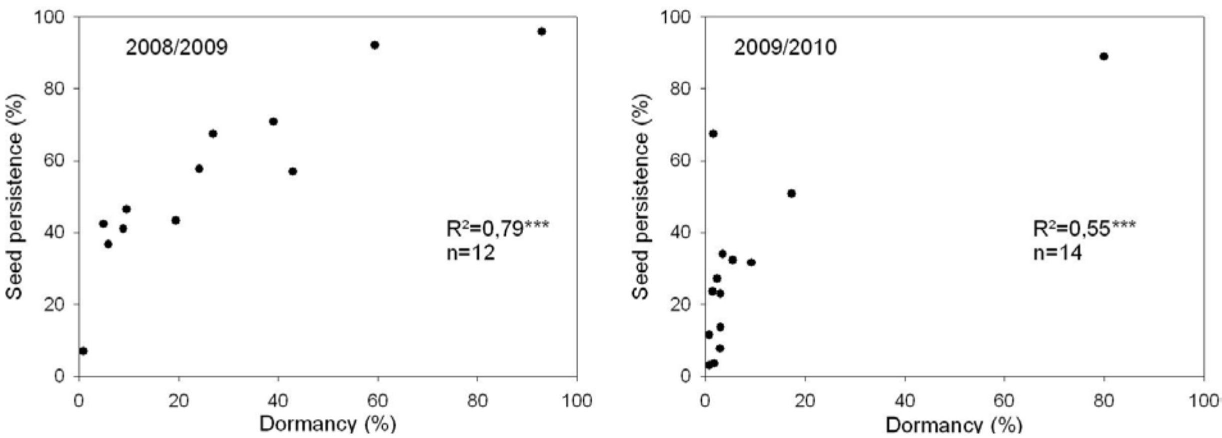

Fig. 2: Correlation between genotype-specific secondary dormancy and seed persistence after 6 months burial at 10 cm soil depth in the seasons 2008/2009 and 2009/2010 (p<0.001).

Although dormancy of all cultivars was below 20 % (except cultivar Smart) in 2009/2010 due to aging during storage, seed persistence in the soil varied from 6 to 63 % (except cultivar Smart: 83 %; Fig. 1 and Fig. 2). Comparable to the 2008/2009 results, no significant differences between GM genotypes and their near-isogenic cultivars could be observed, suggesting that seed ingredients such as resveratrol and reduced sinapine have a negligible effect on seed persistence or dormancy. Notably, the thick-coated cultivar “Thick” with a dormancy of only 2 % exhibited seed persistence of 63 % (Fig. 1 and 2).

To better investigate the potential impact of seed coat characteristics (thickness and crude fiber content) on seed persistence, coefficients of determination between NDF, ADF, ADL and seed persistence were determined for selected cultivars (Tab. 3). There were positive correlations between NDF, ADF, ADL and seed persistence with R^2 values of 0.70 to 0.91. ADF and seed persistence correlated significantly in both years. The correlation between ADL and seed persistence was statistically significant in 2009. NDF and seed persistence did not significantly correlate in either year. In addition to a genotypic predisposition to secondary dormancy, seed coat characteristics may play a role in long term seed persistence in the soil and should therefore be further investigated.

Tab. 3: Correlation between crude fiber fractions and seed persistence in soil of oilseed rape seeds buried at 10 cm soil depth for 6 months.

	Correlations of crude fibre fractions × seed persistence (SP)					
	NDF×SP		ADF×SP		ADL×SP	
	R^2	p	R^2	p	R^2	p
2008/2009	0.73 (n=3)	0.35^{ns}	0.91 (n=4)	0.05*	0.76 (n=4)	0.13^{ns}
2009/2010	0.80 (n=6)	0.11^{ns}	0.75 (n=6)	0.03*	0.70 (n=6)	0.04*

NDF neutral-detergent fibre, ADF acid-detergent fibre; ADL acid-detergent lignin

Soil texture had a significant impact on seed persistence in both seasons (Fig. 4). A significantly smaller number of seeds persisted in lS (2008/2009: 41 %; 2009/2010: 8 %) as compared to sC (2008/2009: 66 %; 2009/2010: 33 %) and cL (2008/2009: 64 %; 2009/2010: 45 %). Soil environmental conditions specific to soil texture, such as temperature and humidity, may have accounted for differing conditions for maintaining or breaking dormancy.

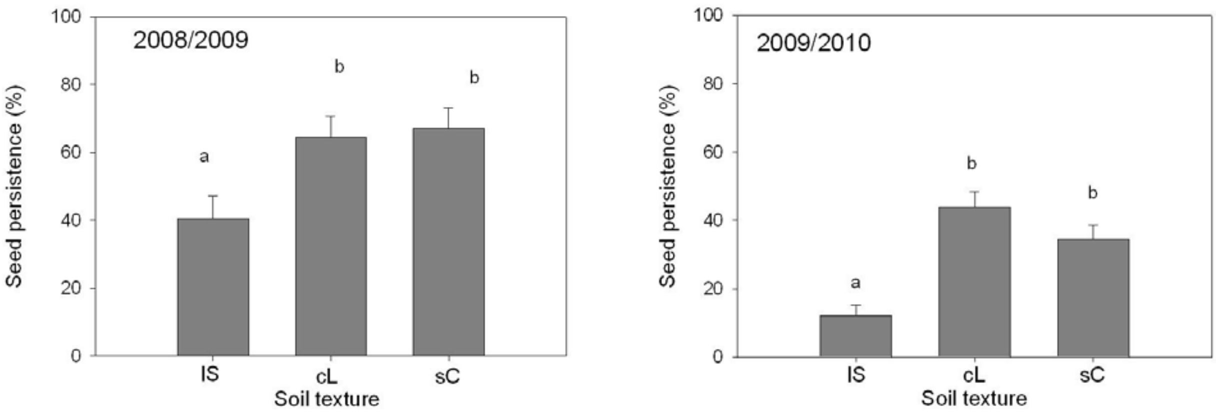

Fig. 4: Seed persistence (percentage of viable seeds after burial of 500 seeds over 6 months in 10 cm soil depth) in three different soils (lS - loamy sand; cL - clay loam, lS) in the experimental seasons 2008/2009 and 2009/2010. Treatments assigned the same letter are not significantly different. Error bars = standard error.

The probability of seed persistence and emergence of volunteers is mainly influenced by the genotypic predisposition to secondary dormancy but may also be an effect of genotypic seed coat characteristics and soil texture. This must be considered if volunteers are to be avoided.

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