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Overcoming seed dormancy in oilseed rape (*Brassica napus L.*) with exogenous compounds

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Summary

Dormant seeds of oilseed rape (OSR) can persist in the soil and cause OSR volunteers in subsequent crops. Several approaches were tested in the laboratory and in the field to determine whether dormancy induction and seed persistence can be reduced by using dormancy-breaking exogenous compounds. In a laboratory experiment, OSR seeds were coated with KNO_3, micronutrients, or gibberellic acid (GA) prior to a secondary dormancy test. In a field experiment, seeds were coated in a manner analogous to the laboratory experiment, and then buried 10 cm deep in the soil for 2.5 months. In a practical demonstration, OSR plants were sprayed with either urea ammonium nitrate (UAN) or a commercial product containing GA prior to seed maturity. Seed coating (laboratory and field experiments) reduced secondary dormancy and seed persistence in the field by up to 99%. The efficiency of the treatments for mitigating secondary dormancy (laboratory and field experiments) in decreasing order was GA > micronutrients > KNO_3 > control. With pre-maturity spraying (practical demonstration), UAN reduced primary dormancy by up to 77% and the development of secondary dormancy by up to 38%; GA had no effect. Dormancy and seed persistence of OSR seeds may be reduced by a pre-maturity UAN treatment of OSR mother plants, or by applying appropriate exogenous compounds to OSR seeds.

Keywords: soil seedbank, phytohormones, dormancy breaking, micronutrients, KNO_3, canola.

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Introduction

Oilseed rape (OSR; *Brassica napus* L.) seeds from harvest losses can develop secondary dormancy when exposed to unfavourable germination conditions, such as osmotic stress or oxygen deficiency in darkness (Momoh *et al.*, 2002). This would be the case when OSR seeds are buried by tillage under dry conditions (Gruber *et al.*, 2004a; Huang *et al.*, 2015). Secondarily dormant OSR seeds form a soil seedbank and are able to emerge as volunteers in following crops for up to 11 years (Andersen *et al.*, 2010). *Brassica napus* volunteers can affect crop yield (Krato & Petersen, 2012), and they are of particular concern if they become weeds in the sown OSR, because selective chemical control can then become difficult, especially when OSR varieties without herbicide-resistant traits are grown. Moreover, volunteers can reduce OSR oil quality and potentially spread herbicide-resistant genes. Apart from secondary (induced) dormancy, OSR seeds can show primary (innate) dormancy to a small extent at harvesting (Momoh *et al.*, 2002; Huang *et al.*, 2016), which rapidly decreases to zero after <6 months of storage (Gruber *et al.*, 2004a).

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Strategies to reduce OSR seed persistence in the soil already exist. By growing OSR varieties that tend to have lower secondary dormancy levels, the likelihood of seed survival in the soil is reduced (Gruber *et al.*, 2009; Huang *et al.*, 2016). In areas with harsh winter conditions, the soil seedbank is decreased by winterkill because emerging OSR volunteers perish as a result of very low temperatures in winter (Geddes & Gulden, 2017). Also, the amount of OSR seeds that enter the soil seedbank can be reduced by delayed stubble tillage in combination with a deep primary tillage (Gruber *et al.*, 2004b, 2010; Huang *et al.*, *2015*).

Seed dormancy is reduced, and germination promoted by nitrate, gibberellic acid ($C_{19}H_{22}O_6$; GA) and micronutrients (Finch-Savage & Leubner-Metzger, 2006; Bojovic, 2010; Farooq *et al.*, 2011; Arefi *et al.*, 2012; ISTA, 2013; Dresch *et al.*, 2014). Therefore, these chemicals seem worthy of further study for the development of additional strategies to avoid a persistent soil seedbank. If seeds from harvest losses drop onto the soil, seeds could theoretically be treated by the application of dormancy reduction agents to avoid secondary dormancy. Also, a reduction in the potential of dormancy development of OSR seeds during seed development on the mother plant would minimise the establishment of a soil seedbank.

The aim of this study was to test: GA, Gibb$_3$® (a GA-containing commercial plant protection product), micronutrient suspensions Wuxal® Terios (WT) and Wuxal® Semilion (WS), urea ammonium nitrate solution (UAN) and potassium nitrate (KNO_3), for their ability to prevent or reduce the ability of OSR seeds to become dormant. Three consecutive trials (one laboratory, one field experiment and one practical demonstration) were set up. First, the efficacies of proposed dormancy reduction agents were determined on mature seeds. Second, as a demonstration of practical applicability, OSR plants were sprayed with dormancy reduction agents during seed development to show their effect on primary and secondary dormancy development. We hypothesised (i) that all tested exogenous compounds would reduce the induction of secondary dormancy, (ii) that different exogenous compounds differ in their efficacy to reduce secondary dormancy induction, (iii) that both low- and high-dormancy varieties would respond to the application of the exogenous compounds, and (iv) that secondary dormancy induction of high-dormancy varieties is more reduced compared with that of low-dormancy varieties.

Material and methods

Plant materials

In all experiments, OSR varieties with different dormancy levels were chosen. Because of time lags between experiments and interest in using contemporary varieties, varieties differed among the three experiments. After harvesting, the seeds were stored in darkness at 5°C with a moisture content of 8% until the start of the experiments. Prior to the experiments, the seeds were inspected visually; only intact seeds were used.

Dormancy tests

To investigate seed persistence as well as primary and secondary dormancy, different testing procedures were used. For the laboratory experiment, secondary dormancy of seeds was tested following the Rapid Dormancy test (Weber *et al.*, 2010). For the practical demonstration, primary dormancy and secondary dormancy of the seeds were tested in accordance with the Hohenheim Standard Dormancy test (Weber *et al.*, 2010). The persistence rate of seeds in the field experiment was determined by a germination test, which is also a subprocedure. It is also a subprocedure of the Rapid Dormancy test and the Hohenheim Standard Dormancy test to examine seeds' viability.

The Rapid Dormancy test was performed by first subjecting seeds to conditions known to induce secondary dormancy in OSR seeds. Specifically, seeds were placed in Petri dishes (diameter: 8.5 cm; 50 seeds per Petri dish) that contained a polyethylene glycol ($HO(C_2H_4O)_nH$; PEG; average molecular weight 5000 g mol^{-1}) solution that generated an osmotic potential of −15 bar (354.4 g PEG in 1 L deionised water). Petri dishes with seeds and PEG solutions were kept in darkness at 20°C for 7 days. Seeds were then subjected to conditions conducive to germination by transferring them in darkness to Petri dishes with a double layer of filter paper and 8 mL of deionised water. Petri dishes with seeds and water were kept in darkness at 20°C for 7 days. Non-dormant seeds germinated during this period. Afterwards, ungerminated seeds were tested for viability for 7 days (12 h darkness and 3°C, 12 h light and 30°C: this environment terminates dormancy and stimulates germination). Viable seeds, which did not germinate prior to the viability test, were defined as secondary dormant seeds. The dormancy potential was then calculated as a percentage; the number of dormant seeds was divided by the total number of viable seeds used in the test.

To investigate secondary dormancy in accordance with the Hohenheim Standard Dormancy test, 100 seeds per laboratory replicate were put in darkness into Petri dishes (diameter: 8.5 cm) fitted with filter paper and filled with 8 mL PEG solution (354.4 g in 1 L H_2O). Afterwards, the seeds were moved in darkness to a germination cabinet for 14 days at 20°C. During this time, germinated seeds and seed losses (mouldy and soft seeds) were counted and removed, according to ISTA (2013). Ungerminated, firm and viable-looking seeds were classified as potentially dormant. For testing actual viability, seeds were exposed to conditions that stimulate germination (7 days under 12 h darkness at 3°C, 12 h light at 30°C). Germinated seeds were counted, and the dormancy potential was then calculated in the same way, as in the Rapid Dormancy test. Primary seed dormancy was assessed with the Hohenheim Standard Dormancy test without the first step that induces secondary dormancy.

Dormancy induction in PEG with pre-treated seeds (Laboratory Experiment)

Seeds of the OSR varieties Lilian and Nemax harvested in 2009 were coated with different agents during May–June 2012. Seeds were stored from harvest until use in darkness at 5°C. Agents evaluated were solutions of KNO_3, gibberellic acid (GA), $Gibb_3$®, WS and WT at different concentrations (Table 1). $Gibb_3$® is a GA-containing growth regulator used in viniculture to improve air circulation within fruit clusters of grape (*Vitis* sp.). Concentration is provided in Table 1. WS and WT are highly concentrated nutrient suspensions for seed treatment of cereals, cotton and rice, which were developed to promote germination and seedling vigour under adverse growing conditions.

To increase the adhesion of agents to seeds, 2 g talcum ($Mg_3Si_4O_{10}(OH)_2$) was added per 100 mL for the KNO_3, GA and $Gibb_3$® solutions. The coating procedure was done as follows: 20 µL (GA-, KNO_3-, $Gibb_3$®, deionised water, deionised water + talcum) or 40 µL (WT, WS) of the solutions was added to Petri dishes containing 50 seeds each. Using forceps, the seeds were then carefully rolled to spread the agents uniformly over the seed surfaces. Coated seeds were dried at room temperature for 24 h. Seeds were then subjected to the Rapid Dormancy test (for method, see description under Dormancy test). Petri dishes (diameter: 8.5 cm) containing the treatments (variety × coating) were arranged in an germination cabinet with four replicates during the dormancy test procedure.

Seed burial in the soil with pre-treated seeds (field experiment)

The field experiment was performed from June to September 2012 at an experimental field (Luvisol, soil type: clay loam) of the University of Hohenheim, Stuttgart, South-West Germany. Seeds from the harvest 2009 (four replicates of 100 seeds per treatment) of the winter OSR varieties Lilian and Nemax from seed coating treatments 2, 3, 4, 6, 7 and 9 (Table 1) were enclosed in 10 × 10 cm fabric mesh bags and buried at 10 cm soil depth on 27 June 2012. The experiment was arranged in a randomised complete block design with four replicates. The mesh size of the bags was 0.5 mm. Prior to and immediately after seed burial, the experimental area (3 × 2 m) was covered by a foil tunnel for 14 and 7 days respectively, permitting the soil to dry, and to provide beneficial conditions for secondary dormancy induction. Precipitation and temperature data for the duration of the field experiment after removal of the foil tunnel are displayed in Fig. 1.

After 2.5 months, the mesh bags were unearthed and intact non-germinated seeds were counted. The obviously intact persisted seeds then underwent a germination test (7 days under 12 h darkness at 3°C, 12 h light at 30°C) to determine whether the seeds are still viable. The persistence rate was calculated as a percentage of intact viable seeds after burial from the initial number of buried seeds.

Dormancy prevention by pre-maturity treatments in the field (practical demonstration)

In the practical demonstration, two varieties of OSR were treated with agents that potentially inhibit dormancy induction. The OSR varieties used were NX2220 (NX) and NT132178H (NT). Applied agents were $Gibb_3$® and urea ammonium nitrate solution (UAN) in two different concentrations per agent (Table 2). The demonstration was a field study conducted in 2015 and repeated in 2016 in an experimental field (Luvisol, soil type: loam) at the agricultural experiment station 'Ihinger Hof' of the University of Hohenheim, Renningen, South-West Germany. OSR was sown on 27 August 2015 and 25 August 2016. For each year, the row spacing was 12 cm and the sowing density was 45 seeds m^{-2}. The agronomic practices during the growing season are listed in Table 3 for each trial year.

The practical demonstration was arranged in a split-plot design with four field replicates. Each main plot (variety) had dimensions of 6 m × 4 m in which the subplots (treatment) of 0.5 m^2 size were distributed randomly. On 22 June 2016 and on 19 June 2017 at

Table 1 Exogenous compound treatments for oilseed rape seeds and their concentrations for a dormancy test (laboratory experiment) and a burial experiment (field experiment)

Treatment		Concentration	Inclusion of talcum	Comment	Source
1	Distilled water (control)	–	No	–	–
2	Control + talcum	2% talcum	Yes	Talcum ($Mg_3Si_4O_{10}(OH)_2$) dissolved in deionised water	Rema AG, Poing, Germany
3	KNO_3	0.4% KNO_3 + 2% talcum	Yes	Dissolved in deionised water	Merck, Düsseldorf, Germany
4	Gibberellic acid (GA) 0.04	0.04% GA + 2% talcum	Yes	Dissolved in deionised water	Merck Schuchardt OHG, Hohenbrunn, Germany
5	GA 0.08	0.08% GA + 2% talcum	Yes	Dissolved in deionised water	
6	$Gibb_3$®	1.0% $Gibb_3$ (=0.1% gibberellic acid) + 2.0% talcum	Yes	Commercial product, contains 10% Gibberellic acid dissolved in deionised water	Globachem Sint-Truiden, Belgium
7	Wuxal® Terios (WT 100)	Pure (100%)	No	Pure suspension, commercial product, contains 25 g Cu, 15 g Mn, 5 g Mo, 25 g Zn, 106 g NH_4-N, 150 g P_2O_5, 21 g S per litre	Wilhelm Haug GmbH & Co. KG, Düsseldorf, Germany
8	Wuxal® Terios (WT 50)	50.0%	No	Dissolved in deionised water	
9	Wuxal® Semillion (WS 100)	Pure (100%)	No	Pure suspension, commercial product, contains 22.5 g N, 39 g S, 15 g B, 7.5 g Cu, 15 g Mn, 22.5 g Mo, 22.5 g Zn per litre	Wilhelm Haug GmbH & Co. KG, Düsseldorf, Germany
10	Wuxal® Semillion (WS 50)	50.0%	No	Dissolved in deionised water	

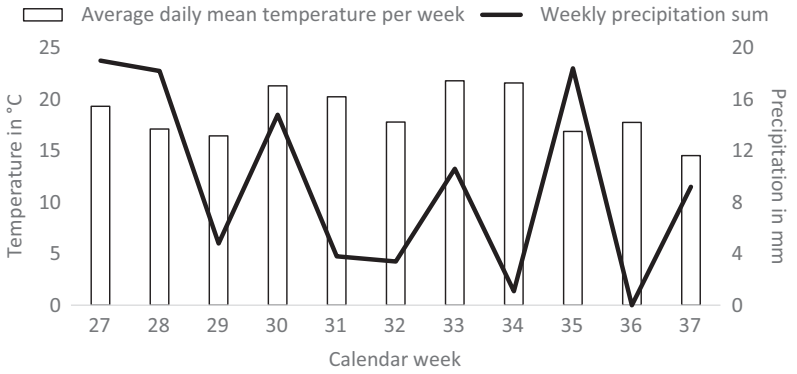

Fig. 1 Average daily mean temperature in °C and weekly precipitation sum in mm for the duration of the field experiment at the University of Hohenheim after foil tunnel removal (calendar weeks in 2012).

OSR stage BBCH 77 (seed development), agents were applied by spraying with a mechanical hand-held sprayer in accordance with the application rates in Table 2. After OSR reached maturity, the upper parts of OSR plants with pods were collected manually on 19 July 2016 and on 17 July 2017. OSR plant materials were dried separately for each plot in cotton sacks at room temperature for 1 day. Afterwards, the harvested material was threshed and winnowed to isolate intact seeds without impurities. From July to August, the seeds underwent a laboratory experiment for testing primary and secondary dormancy in accordance with the Hohenheim Standard Dormancy test. From the harvested seed sample of one field replicate, four laboratory replicates of the Standard Dormancy test were sub-sampled.

Statistical analysis

Statistical analyses for all experiments were conducted in accordance with the respective experimental designs using the procedure MIXED of the software package

Table 2 Exogenous compound treatments at seed development stage of oilseed rape plants and their application rates to test their impact on primary and secondary dormancy

Treatment		Application rate	Comment	Source
1	Control	–	Without treatment	–
2	Urea ammonium nitrate solution low concentration (UANl)	80 mL 2.78% UAN solution m^{-2}	Equals 8 kg N ha^{-1}	SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt Wittenberg, Germany
3	Urea ammonium nitrate solution high concentration (UANh)	80 mL 27.78% UAN solution m^{-2}	Equals 80 kg N ha^{-1}	
4	$Gibb_3$® low concentration ($Gibb_3$ l)	80 mL 0.002% $Gibb_3$® solution m^{-2}	Equals 1.6 g Gibberellic acid ha^{-1}	Globachem Sint-Truiden, Belgium
5	$Gibb_3$® high concentration ($Gibb_3$ h)	80 mL 0.02% $Gibb_3$® solution m^{-2}	Equals 16 g Gibberellic acid ha^{-1}	

Table 3 Agronomic treatments during the growing season of the practical demonstration

Date	Treatment	Active agent	Trade name/manufacturer
Trial year 2016			
31 August 2015	Herbicide	500 g ha^{-1} metazachlor 500 g ha^{-1} dimethenamid 250 g ha^{-1} quinmerac	Butisan® Gold/BASF
08 October 2015	Herbicide	80 g ha^{-1} prapaquizafop	Agil®-S/Adama
26 October 2015	Fungicide	96 g ha^{-1} prothioconazole 192 g ha^{-1} tebuconazole	Tilmor®/Bayer CropScience
15 March 2016	Nitrogen fertiliser	Ammonium sulphate nitrate (90 kg N ha^{-1})	Domogran® 45/Domo Chemicals
05 April 2016	Fungicide	56 g ha^{-1} prothioconazole 112 g ha^{-1} tebuconazole	Tilmor®/Bayer CropScience
05 April 2016	Insecticide	57.5 g ha^{-1} etofenprox	Trebon® 30 EC/BASF
07 April 2016	Nitrogen fertiliser	Ammonium sulphate nitrate (90 kg N ha^{-1})	Domogran® 45/Domo Chemicals
11 April 2016	Insecticide	72 g ha^{-1} thiacloprid	Biscaya®/Bayer CropScience
22 April 2016	Insecticide	40 g ha^{-1} acetamiprid	Mospilan® SG/Cheminova
Trial year 2017			
01 September 2016	Herbicide	500 g ha^{-1} metazachlor 500 g ha^{-1} dimethenamid 250 g ha^{-1} quinmerac	Butisan® Gold/BASF
05 October 2016	Insecticide	0.075 g ha^{-1} Beta-Cyfluthrin	Bulldock®/Adama
05 October 2016	Herbicide	80 g ha^{-1} prapaquizafop	Agil®-S/Adama
05 October 2016	Herbicide	93.5 g ha^{-1} clopyralid 23.5 g ha^{-1} picloram	Effigo™/Dow AgroSciences
21 October 2016	Nitrogen fertiliser	Calcium ammonium nitrate (30 kgN ha^{-1})	YaraBela® EXTRAN 27®/Yara International
15 March 2017	Nitrogen fertiliser	Ammonium sulphate nitrate (80 kg N ha^{-1})	Domogran® 45/Domo Chemicals
03 April 2017	Fungicide	48 g ha^{-1} prothioconazole 96 g ha^{-1} tebuconazole	Tilmor®/Bayer CropScience
03 April 2017	Insecticide	57.5 g ha^{-1} etofenprox	Trebon® 30 EC/BASF
06 April 2017	Nitrogen fertiliser	Ammonium sulphate nitrate (90 kg N ha^{-1})	Domogran® 45/Domo Chemicals

SAS 9.3 (SAS Institute, Cary, NC). In the four experiments, different fixed effects, random effects and experimental designs were set up (Table 4).

In the laboratory and the field experiments, a transformation of data was necessary to normalise variances. The following arcsine-transformation, according to Chatterjee and Hadi (2012), was used:

$$y = \arcsin\left(\sqrt{\frac{d+\frac{3}{8}}{v+\frac{3}{4}}}\right), \quad (1)$$

where y = transformed value, d = number of dormant seeds and v = number of viable seeds for the laboratory experiment and y = transformed value,

Table 4 Assumption for model parameters and experimental design of the experiments

Experiment	Fixed effects	Random effects	Design
Laboratory	Seed coating treatment, replicate (separate analysis per variety)	Error	Randomised complete block design
Field	Seed coating treatment, replicate (separate analysis per variety)	Error	Randomised complete block design
Practical demonstration	Maternal plant treatment, field replicate (separate analysis per variety)	Field replicate * laboratory replicate error	Randomised complete block design

d = number of persisted seeds and v = number of buried seeds for the field experiment.

In the practical demonstration, a data transformation was not necessary. For the laboratory experiment, the response variable was the secondary dormancy level of OSR seeds. In case of the field experiment, the response variable was the persistence level of OSR seeds. For the practical demonstration, the response variables were primary and secondary dormancy levels of the OSR seeds. The comparison of means was done within variety to highlight the efficacy of exogenous compounds within single varieties. Residuals were checked graphically for homogeneity and normal distribution. If the factor seed coating treatment within 1 year and one variety was identified to be significant at $\alpha = 0.05$, means were compared using the Student's t-test. For presentation purposes, means and standard errors of means were back-transformed after statistical analysis.

Results

Laboratory experiment

Coating of OSR seeds with potential dormancy-breaking or germination-promoting agents significantly reduced the induction of secondary dormancy (Fig. 2). The high-dormancy variety Lilian tended to show higher secondary dormancy than the variety Nemax in all treatments except those with GA. In both varieties, talcum had no effect on secondary dormancy induction compared with the control. While KNO_3 and WS 50 led to a significant reduction in the induction of secondary dormancy in Nemax, the secondary dormancy level of Lilian was not affected by KNO_3 or WS 50. WS 100 followed by both concentrations of WT resulted in a significant reduction in secondary dormancy in both varieties. Treatments containing gibberellic acid ($Gibb_3$® and GA) lowered secondary dormancy induction of both varieties even further to below 5%. The secondary dormancy induction of Nemax was 43% in the control and was reduced, depending on treatment, by between 54% and 97%.

Field experiment

After the burial period, 97% of seeds were intact and non-germinated (data not shown). The rates of seed viability after burial did not differ between varieties (data not shown). Coating the seeds with any of the agents reduced the seed persistence of both OSR varieties over 3 months compared with untreated seeds (Fig. 3). While *c*. 40% of the seeds of Lilian and 14% of Nemax survived without germinating in the non-treated control, <17% (Lilian) or 2% (Nemax) of the seeds persisted when treated with KNO_3, and seed persistence was generally below 3% for both varieties when seeds were coated with WS 100, WT 100, $Gibb_3$® and GA 0.04 before burial (Fig. 3).

Practical demonstration

In the trial year 2016, the levels of primary dormancy across varieties and treatments ranged between 3.3% [treatment with urea ammonium nitrate solution high concentration (UANh) of variety NT] and 14.5% (control, NT; Fig. 4A). Pre-harvest treatment had a significant effect on the level of primary dormancy. Only the treatment UANh resulted in primary dormancy significantly lower than the control (48.8% lower for NX and 77.2% lower for NT; Fig. 4A). No significant differences occurred across the pre-harvest treatments in the trial year 2017 (Fig. 4B). The level of primary dormancy ranged between 2.9% [treatment with urea ammonium nitrate solution low concentration (UANl) of variety NX] and 0.1% (UANl, NT; Fig. 4B).

In both trial years, all pre-harvest treatments in NX had no significant impact on the development of secondary dormancy (Fig. 5A and B). All treatments in NT resulted in lower dormancy values than all treatments in NX. In NT, only the pre-harvest treatment UANh (42.1% in trial year 2016; 28.8% in trial year 2017) showed a significant lower value than the

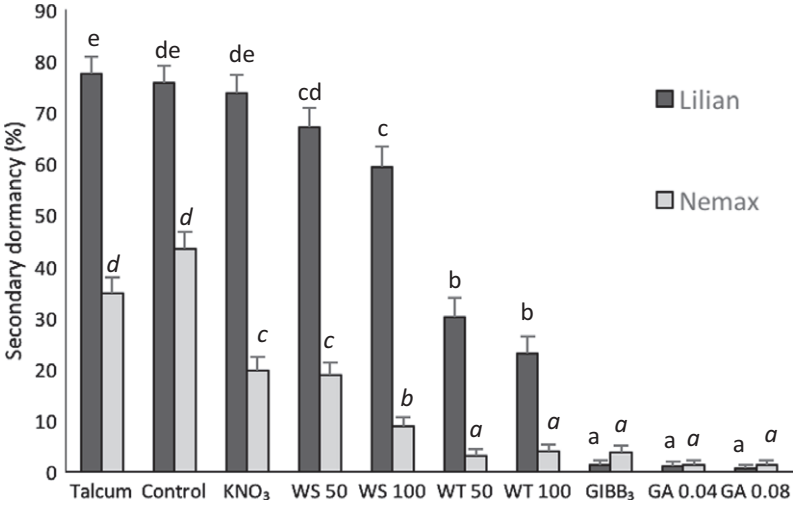

Fig. 2 Effect of seed coating on secondary dormancy induction of two oilseed rape varieties (Lilian and Nemax) with different agents. Values within one variety labelled by the same letter are not significantly different ($\alpha = 0.05$; Student's *t*-test on transformed values; letters for Nemax in italics). Error bars: standard error of mean. KNO_3: 0.4% solution; WS: Wuxal® Semillion pure (100) and diluted to 50% (50); WT: Wuxal® Terios pure (100) or diluted to 50% (50); $Gibb_3$®: 0.1% solution of a gibberellic acid containing (10%) growth regulator; GA: gibberellic acid in 0.04% (0.04) and 0.08% (0.08) concentration (Table 1).

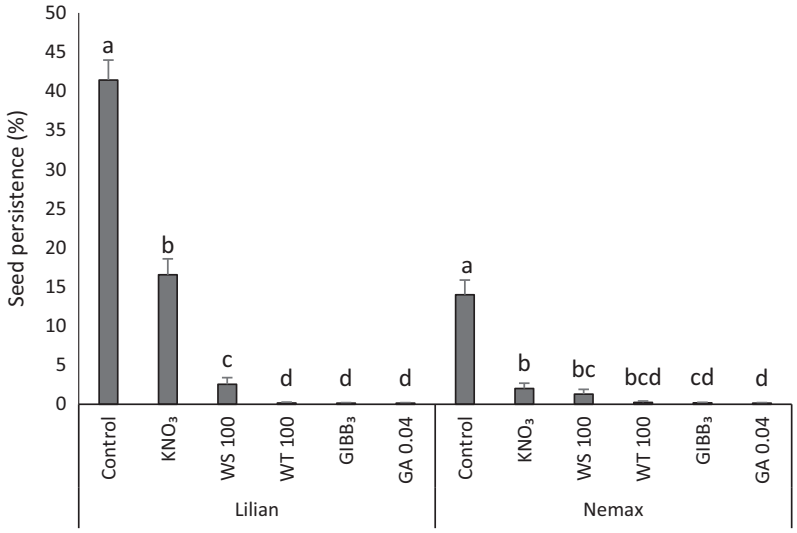

Fig. 3 Seed persistence of buried seeds of two oilseed rape varieties (Lilian and Nemax), coated with different agents prior to burial, after a period of 3 months buried at 10 cm soil depth in a field at University of Hohenheim, 2012. Values within one variety labelled by the same letter are not significantly different ($\alpha = 0.05$; Student's *t*-test on transformed values). Error bars: standard error of mean. KNO_3: 0.4% solution; WS 100: Wuxal® Semillion pure; WT 100: Wuxal® Terios pure; $Gibb_3$®- 0.1% solution of a gibberellic acid containing (10%) growth regulator; GA 0.04: gibberellic acid in 0.04% concentration (Table 1).

untreated control (67.7% in trial year 2016; 46.8% in trial year 2017) which also was lower than the other treatments.

Discussion

Secondary dormancy was reduced when OSR seeds were treated with exogenous gibberellic acid (GA), potassium nitrate (KNO_3) and micronutrients. GA plays an important role in releasing seeds from dormancy and promoting germination of imbibed seeds (Arefi *et al.*, 2012; ISTA, 2013; Shu *et al.*, 2015). KNO_3 is also known to be capable of breaking dormancy (Baskin & Baskin, 2001). The dormancy-breaking effect can be linked to nitrate (Baskin & Baskin, 2001; Finch-Savage & Leubner-Metzger, 2006) and is presumably caused, in part, by the reduction in abscisic acid levels along with an increase in GA levels in the seeds of several crop species (Matakiadis *et al.*, 2009; Shu *et al.*, 2015). In addition, it should be noted that extended storage time might have reduced potential for secondary dormancy reduction in OSR seeds (Gulden *et al.*, 2004).

In the laboratory experiment, the high-dormancy variety Lilian and the low-dormancy variety Nemax responded differently to the KNO_3 treatment, although seed persistence of both varieties was reduced by KNO_3 in the field experiment. In the laboratory, variety-specific responses to KNO_3 might have been caused by the relatively short duration of KNO_3 exposure (2 weeks). In the field experiment, seeds potentially interacted with KNO_3 for 2.5 months. The relatively long period of KNO_3 exposure under field conditions might have facilitated KNO_3 effects on seeds for both high- and low-dormancy varieties.

Coating seeds with micronutrient solutions reduced secondary dormancy induction somewhat, depending on the variety, especially if the solutions were highly

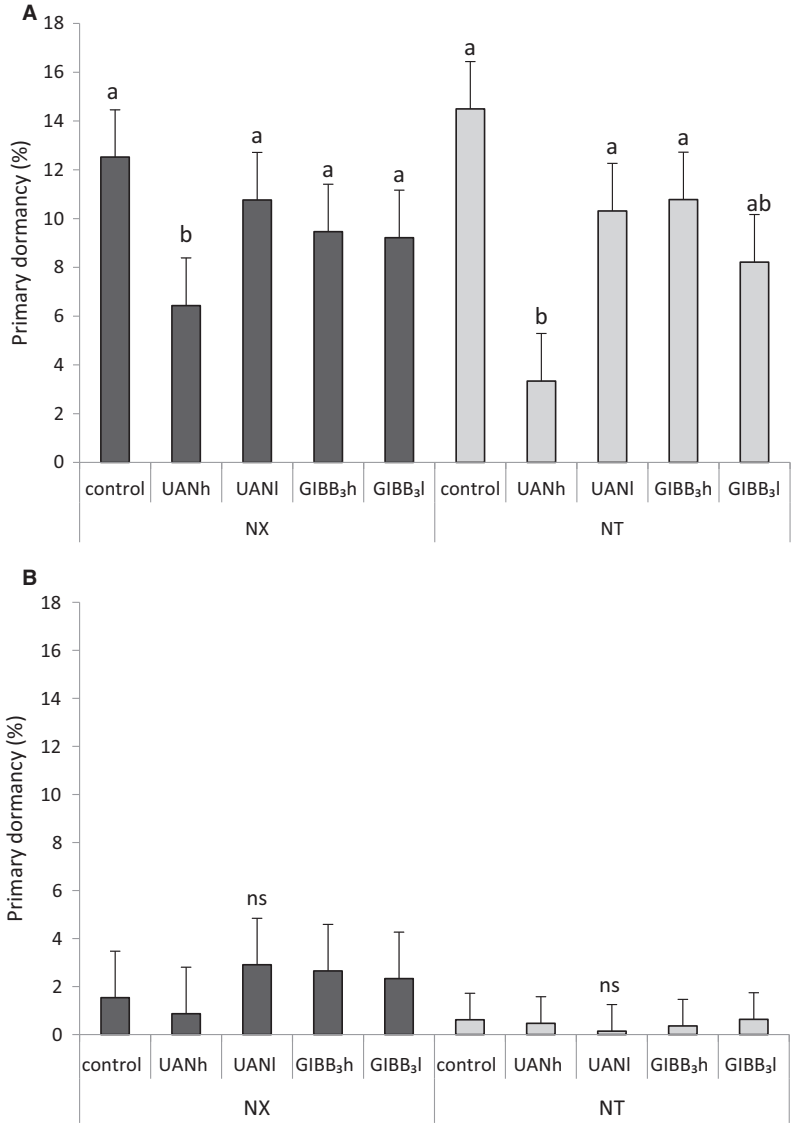

Fig. 4 Primary dormancy of oilseed rape seeds of two varieties (NX and NT) after pre-harvest treatment of plants with different agents of the trial years 2016 (A) and 2017 (B). Values within one variety labelled by the same letter are not significantly different ($\alpha = 0.05$; Student's *t*-test). Error bars: standard error of mean. Control: no treatment; UANh: treatment with 80 mL 27.78% UAN solution m^{-2}; UANl: treatment with 80 mL 2.78% UAN solution m^{-2}; $GIBB_3$ h: treatment with 80 mL 0.02% solution of $Gibb_3$® a gibberellic acid containing (10%) growth regulator m^{-2}; $GIBB_3$l: treatment with 80 mL 0.002% solution of $Gibb_3$® m^{-2} (Table 2).

concentrated. The mode of action, however, is not yet clear. Some micronutrients are known to promote germination, for example boron, copper, zinc and manganese (Cresswell & Nelson, 1972; Delatorre & Barros, 1996; Farooq *et al.*, 2012; Imran *et al.*, 2015). The tested solutions WS and WT contained nitrogen and other macronutrients, so solution components other than the micronutrients could also have altered dormancy responses. Seeds coated with micronutrients responded more strongly to the treatment when tested in the field than when tested in the laboratory. It is possible that temperature fluctuations and other external factors unique to field conditions prevented secondary dormancy induction more in the field than under artificial, controlled conditions in the laboratory.

Spraying agents before ripening on the standing crop could be an approach to reduce primary dormancy, and also the capacity of seeds to become secondarily dormant. It was tested in a first approach, in the field with selected agents and selected concentrations (practical demonstration), so as to show whether there is any response in dormancy at all. Both primary (in the first trial year) and secondary dormancy (in both trial years) could be reduced. In the second trial year, the general primary dormancy level was relatively low, thus a significant decreasing effect of the agents could not be observed. Primary dormancy development is mainly regulated by abscisic acid (Nambara *et al.*, 2010). Its synthesis is determined genetically and by environmental conditions (Chono *et al.*, 2006), which could have caused different primary dormancy levels in both trial years.

Treating plants with dormancy reduction agents could be adopted in farming practice by usage of existing spraying technologies. Ecological harm due to

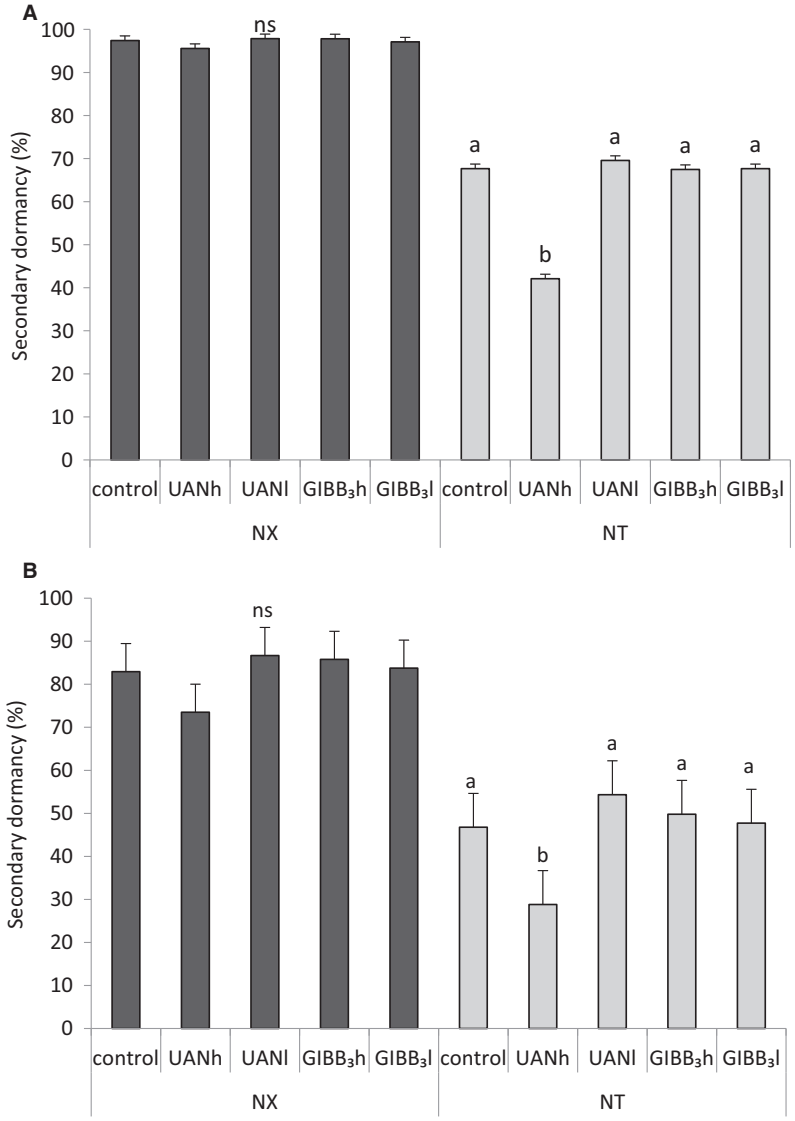

Fig. 5 Secondary dormancy of oilseed rape seeds of the varieties NX and NT after pre-harvest treatment of plants with different agents of the trial years 2016 (A) and 2017 (B). Values within one variety labelled by the same letter are not significantly different ($\alpha = 0.05$; Student's *t*-test). Error bars: standard error of mean. Control: no treatment; UANh: treatment with 80 mL 27.78% UAN solution m^{-2}; UANl: treatment with 80 mL 2.78% UAN solution m^{-2}; $GIBB_3$ h: treatment with 80 mL 0.02% solution of $Gibb_3$® a gibberellic acid containing (10%) growth regulator m^{-2}; $GIBB_3$ l: treatment with 80 mL 0.002% solution of $Gibb_3$® m^{-2} (Table 2).

applications of dormancy reduction agents is unlikely because, in Germany, $Gibb_3$® is a state-approved plant protection product, UAN and KNO_3 are common nitrogen fertilisers, and WS and WT contain micro- and macronutrients that are part of usual fertilisation strategies. However, this study was conducted to examine general effects of exogenous compounds on seed dormancy of OSR. Further investigations are recommended for evaluations of ecological risk and for determining the influence of dormancy reduction agents on seed quality parameters including oil quality.

Primary dormancy can occur in pre-mature seeds during seed development on the mother plant of OSR (Huang *et al.*, 2016). A reduction in primary seed dormancy would be beneficial for managing volunteer OSR, in case of natural pod shatter or pod damage caused by heavy rainfall or hailstorms before harvesting. Studies with *Arabidopsis thaliana* L. indicated that an increase in nitrate availability during seed development may reduce abscisic acid levels in the seed and, therefore, also reduce the dormancy potential (Alboresi *et al.*, 2005; Matakiadis *et al.*, 2009). We do not yet know at which stage the application of UAN, and which concentrations of UAN, would provide the greatest efficacy. $Gibb_3$® did not affect primary and secondary dormancy in the practical demonstration. Exogenous applications of $Gibb_3$® might not affect OSR seed dormancy or the chosen spraying scheme

could have prevented its efficacy. Specifically, the spraying scheme might not have facilitated GA uptake by the plants, or the concentration, or the timing, was not yet appropriate.

Although the experiments showed that seed dormancy in OSR can be manipulated readily through the application of effective exogenous compounds, procedures have to be developed and specified to show how these compounds should be applied in the field, in terms of timing, concentration, frequency and techniques. This study, however, provided basic information about the effects of the tested substances and gave deeper insights into strategies showing how the development of dormancy may be prevented in OSR seeds. The study points out several options for practical farming, which may serve in the future as further strategies to overcome the unwanted effects of OSR volunteers.

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