Institute of Crop Science

Universität Hohenheim

Department: Agronomy

Prof. Dr. Wilhelm Claupein

Management of volunteers derived from imidazolinone-tolerant oilseed rape

Dissertation
In fulfillment of the requirements for the degree
"Doktor der Agrarwissenschaften"
(Dr. sc. agr. / Ph. D. in Agricultural Sciences)
submitted to the
Faculty of Agricultural Sciences
by Shoubing Huang
born in Shandong, China

March 2016

This thesis was accepted as a doctoral dissertation in fulfillment of the requirements for the degree "Doktor der Agrarwissenschaften" (Dr. sc. agr. / Ph.D in Agricultural Sciences) by the Faculty of Agricultural Sciences at the University of Hohenheim on 8th June 2016.

Date of oral examination: 30th June 2016

Examination Committee

Supervisor and Reviewer:	Prof. Dr. Wilhelm Claupein
Co-reviewer:	Prof. Dr. Jan Petersen
Additional examiner:	PD Dr. Regina Belz
Head of the Committee:	Prof. Dr. Markus Rodehutscord

Table of contents

List of Tables I

List of Acronyms I

1 General introduction 1

2 Publications 10

3 Chapter I 11

Dynamics of dormancy during seed development of oilseed rape (*Brassica napus* L.)

4 Chapter II 21

Seed dormancy in F1 and F2 generations of imidazolinone-tolerant oilseed rape at different locations

5 Chapter III 32

Field history of imidazolinone-tolerant oilseed rape (*Brassica napus*) volunteers in following crops under six long-term tillage systems

6 General discussion 41

7 Summary 57

8 Zusammenfassung 60

9 Reference 64

Acknowledgements 71

Curriculum Vitae 72

List of Tables

Table 1. The most important outcomes and the most important remaining questions that will be focused in the next steps.

List of Acronyms

ABA	abscisic acid
ALS	acetolactate synthase
BnaDOG1	DELAY OF GERMINATION 1 gene in *Brassica napus*
ca.	circa
CL	Clearfield®
DOG1	DELAY OF GERMINATION 1 gene
e.g.	*exempli gratia*, for example
et al.	et alia, and others
EU	European Union
GA	gibberellic acid
GM	genetically modified
HET_IT	heterozygous imidazolinone-tolerant
HOM_IT	homozygous imidazolinone-tolerant
i.e.	*id est*, that is
MD	morphological dormancy
MPa	megapascals, units of water potential
non-CL	non-Clearfield®
non-GM	non-genetically modified
OSR	oilseed rape
PD	physiological dormancy
PM_1	point mutation 1
PM_2	point mutation 2
PY	physical dormancy
KNO_3	potassium nitrate

1 General introduction

1.1 Importance of conventional and herbicide-tolerant oilseed rape

Oilseed rape (OSR, *Brassica napus* L.) is an amphidiploid species (AA×CC, n=19) from the cross between *Brassica campestris* L. (AA, n=10) and *Brassica oleracea* L. (CC, n=9; U, 1935). Nowadays, OSR has become the second most important oilseed crop after soybean in modern agriculture around the world, producing 67.45 million tonnes of seed yield and providing 13.8% of world supply of oilseeds with cultivated area of 31.7 million hectares (FAO, 2015). An increasing demand for OSR would expect due to protein meals/cakes used in animal feed and vegetable oils/fats for biodiesel and human consumption. Besides, OSR is an important part of crop rotations with a high proportion of cereals. In the crop rotation, OSR can contribute to crop diversity, covers the soil for almost a whole year; after harvest, straw of OSR with a comparatively narrow C/N ratio is left on the soil surface, which decomposes fast and provide nitrogen for the following crops. Globally, OSR is grown as spring or winter forms with different vernalization requirement. In Europe, winter OSR is grown widely due to its high seed yield and the climatic conditions.

The large-scale cultivation of OSR was not realised until 1980s due to high levels of glucosinolates and erucic acid of the seeds, which are adverse substances for human health. Breeding and development of double-low varieties (00-varieties; low content of glucosinolates and erucic acid) made by-products of OSR suitable for both human and livestock consumption (Booth & Gunstone, 2004; Thiyam-Hollände et al., 2012).With the advance in plant breeding, new varieties are continuously marketed in terms of oil content and components, yield increase, insect-tolerance, and **herbicide-tolerance** being the most important trait. Tolerance to herbicides for OSR has been developed first by genetic engineering, namely tolerance to the non-selective herbicides glyphosate and to glufosinate-ammonium. OSR varieties with these tolerance are widely grown in the United States of America, Canada, and Australia, but their cultivation is currently not permitted in the European Union due to legal restrictions. Another, non-GM approach for herbicide-tolerant OSR is tolerance to **imidazolinone herbicides** such as imazamox (Krato et al., 2012). They are not restricted by a threshold, and are commercially used in Europe labelled as **Clearfield®** oilseed rape (CL OSR).

The introduction of CL OSR in Europe has raised concern in weed control in crop rotations

with OSR due to **volunteer OSR**. These volunteers result from large harvest losses and high **seed dormancy**, and their chemical control could become difficult if they are tolerant to a group of herbicides.

1.2 Issues in the production of OSR

1.2.1 Seed dormancy of OSR

Seed dormancy is a trait that can prevent germination of a viable seed during unfavourable seasons or under temporarily favourable conditions (Bewley 1997). The definitions and classifications of seed dormancy are different in previous studies. Based on the fact that dormancy is controlled by morphology, physiology or environment of the seed, one classification including five types of dormancy is summarized here (Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006). They are (1) physiological dormancy (PD) resulting from inhibitor chemicals such as abscisic acid (ABA) which can retard embryo growth, (2) morphological dormancy (MD) associated with underdeveloped embryo, (3) morphophysiological dormancy (MPD) combing both PD and MD, (4) physical dormancy (PY) as a result of palisade layers in the fruit or seed coat, and (5) combinational dormancy (PY + PD). Most crop species show non-deep PD which can be broken by stratification, gibberellic acid (GA) application, or release during after-ripening; their seed dormancy is mainly associated with embryo growth and seed coat impermeability to water (Arc et al., 2013).

In OSR, seed dormancy is simply divided into **primary dormancy** and **secondary dormancy** in literatures (Schlink, 1993; Pekrun et al., 1997) as did in *Arabidopsis thaliana* (Hilhorst, 1995). Primary dormancy is supposed to be related to MD, PD or MPD depending on growth stage of seed development, and cannot be induced after harvest. Secondary dormancy in OSR is induced particularly after harvest by external environmental conditions such as dryness, darkness, or oxygen deficiency (Pekrun et al., 1997, 1998). Primary dormancy level in mature OSR seeds has previously been shown to be low (Momoh et al., 2002; Gruber et al., 2004a; Gulden et al., 2004b) or even nearly absent (Schlink 1993). Thus, secondary dormancy turns out to be the main contributor to the seed persistence in the soil (Pekrun et al., 1998; Gulden et al., 2003, 2004b), interacting with varietal effects. Based on the predisposition to secondary dormancy (potential dormancy; seeds with this potential can be induced into secondary dormancy easily) tested by Hohenheim Standard Dormancy Test in laboratory (Weber et al. 2010), 44 OSR

varieties were classified into high (>40%; dormancy level), medium (20-40%), and low (<20%) dormant group in Germany (Gruber et al., 2009). Also, varied dormancy between varieties was reported in Chinese, western European, and Canadian OSR (Momoh et al., 2002; Gulden et al., 2004b).

In addition to varietal effects, maternal environment (e.g. air humidity and temperature) that surrounds mother plants during growth period could influence dynamics of dormancy during seed development in many crop species (King, 1993; Romagosa et al., 2001; Chono et al., 2006), but evidence in OSR is still limited. There appears to be a link between potential secondary dormancy and growing year of OSR (Gruber et al., 2009), which was also detected in the studies of Schatzki et al. (2013a) and Gulden et al. (2004b). With seed development, primary dormancy in OSR decreases probably as a consequence of decrease in seed ABA content, accompanied by an increase of potential secondary dormancy (Haile and Shirtliffe, 2014). Yet, the underlying mechanisms in dormancy dynamics especially for potential secondary dormancy has not been well understood. After harvest, both primary dormancy and potential secondary dormancy decline over time during seed storage, associated with storage conditions (Totterdell and Roberts, 1997; Kebreab and Murdoch, 1999; Gulden et al., 2004b).

Once OSR seeds were induced into secondary dormancy and were buried in the soil by tillage, some of them can persist in the soil over 10 years (Lutman et al., 2003), probably as a consequence of deep dormancy level, slow dormancy release and steady soil conditions. Secondary dormancy of OSR can be broken by alternating light and temperature in a short period (Weber et al., 2010), whereas the speed of release from dormancy especially when seeds are buried in the soil is particularly not understood.

1.2.2 Harvest loss

Large seed losses can occur before and during harvest of OSR as a consequence of pod shattering. The amount of lost seeds ranges from less than 2000 to more than 10,000 seeds m^{-2} (Lutman et al., 2005), depending on the harvesting methods and conditions shortly before and during harvest (Price et al., 1996; Irvine and Lafond, 2010; Zhu et al., 2012) and on varieties (Gan et al., 2008; Cavalieri et al., 2014).

Combine harvesting is widely used for OSR around the world, and windrowing is also used in some regions to minimize harvest seed loss like in Canada, or to allow double cropping

in one year such as in the south of China. Early windrowing OSR can provide the next crop with a longer growing period (Zhu et al., 2012). For these two methods, timing of the operation is important. Full seed maturity is required for the direct combine harvesting, but it is often accompanied by a large harvest loss (Thomas et al., 1991). Early windrowing can reduce seed loss but reduces seed yield and quality (Irvine and Lafond, 2010; Haile et al., 2014). Pod sealant (e.g., chemicals which keep the pots closed) could be a solution for reducing harvest loss due to the reduced pod shattering for the harvest method of windrowing (Nunes et al., 2015), but possible chemical residual have to be taken into account.

Moreover, adverse environmental conditions such as wind, rainfall, or hailstorm before harvest can increase seed loss (Price et al., 1996), and cool and moist weather conditions during harvest can reduce the number of lost seeds due to a high resistance to pod shattering. To date, many breeding approaches have been attempted to improve shatter resistance of OSR (e.g., interspecific hybridization or resynthesis using shatter-resistant species), but the advance still remains limited (Morgan et al., 2000; Hossain et al., 2011). Yet, even in ideal harvest conditions and with suitable harvest methods, the harvest loss in OSR would be still huge, reflecting a challenge in controlling harvest losses.

1.2.3 Tillage and soil seed bank

Soil tillage is the traditional method for weed control in arable fields. Lost crop seeds and weed seeds in the soil or on the soil surface can be vertically and horizontally distributed, depending on tillage intensity and tillage types (Colbach et al., 2000; Mohler et al., 2006). Reduced tillage tends to increase the soil seed bank (Vanasse and Leroux, 2000; Cardina et al., 2002; Sosnoskie et al., 2006; Ruisi et al., 2015). Mouldboard ploughing can bury weed seeds from the soil surface into deep soil layers, whereas shallow soil disturbance by tine cultivator, harrows or rototiller mainly mixes seeds within upper soil layers (Swanton et al., 2000; Gruber et al., 2005; Mohler et al., 2006). Under no-till conditions, medium or high seed numbers were also found near the soil surface (0-5 cm) in some weed species (Cardina et al., 2002; Sosnoskie et al., 2006; Légère et al., 2011). Irrespective of seed distribution by tillage, a general difference between inversion tillage and non-inversion tillage on the numbers of weed seeds in the soil is not obvious or at marginal significance (Cardina et al., 2002; Plaza et al., 2011; Ruisi et al., 2015). The same situation was also found in OSR by Pekrun et al. (2006). However, an obvious difference between tillage

modes in soil seed bank of OSR was found, but which only occurred in the first following year (Gruber et al., 2007, 2010). The variability and contradiction among studies in impacts of tillage modes on soil seed bank size might attribute to different experimental locations.

Taken together, a soil seed bank from OSR seeds can be established due to a large harvest loss and high seed dormancy (particularly secondary dormancy) after harvest of OSR. For the tillage effects, timing of tillage seems more responsible for the build-up of a soil seed bank compared to the mode of tillage. Early incorporation of OSR seeds even by shallow stubble tillage after harvest of OSR can lead to a large soil seed bank (Gruber et al., 2005; Pekrun et al., 2006), probably as a result of fast induction of secondary dormancy by drought, darkness, or oxygen deficiency when seeds are buried in the soil (Pekrun et al., 1997, 1998). Some OSR varieties display relatively high primary dormancy (ca. 15%) in freshly harvested seeds (Gruber et al., 2004a; Haile and Shirtliffe, 2014), which can also increase seed bank size if they are buried immediately after harvest. On the other hand, predisposition to secondary dormancy (potential secondary dormancy tested in the laboratory; Weber et al., 2010) of OSR declines over time during storage, which is associated with storage conditions such as alternating environmental conditions (Gulden et al., 2004b). Similar to this, seeds on the soil surface in a field are exposed to variable environmental conditions, and probably have a faster decline in potential secondary dormancy, and a reduced chance to acquire secondary dormancy as well. A small soil seed bank of OSR was also found in no-till treatment in the subsequent crops after OSR (Gruber et al., 2004a), depending on environmental conditions post-harvest (Gruber et al., 2004b). Environmental conditions preventing OSR seeds from falling dormancy seem more important for obtaining a small soil seed bank than conditions which trigger germination when seeds drop on the soil surface (Pekrun et al., 2005, 2006). However, results in dormancy dynamics of OSR and their interaction with environmental conditions during the period between harvest and the first subsequent tillage are still particularly limited.

The soil seed bank of OSR can persist for several years due to secondary dormancy, but seed bank size declines rapidly during the first few months after OSR, and then slows down in subsequent seasons (Lutman et al., 2003, 2005; Gruber et al., 2004a; Soltani et al., 2013). This decline in seed numbers depends on variety and location. Gulden et al. (2003) reported that in Canada high dormancy spring OSR varieties resulted in about 6- to 12-fold greater seed persistence than low dormancy varieties. Gruber et al. (2010) found that 60% of

initially buried seeds of a high dormancy winter OSR variety (91% potential secondary dormancy from laboratory analyses) were still viable after 4.5 years burial in the soil, while only 8% of a low dormancy variety (9% dormancy) were survival. The evidence on the effects of location on seed persistence is scarce, but seed persistence in dry soil condition seems to be longer than in moist conditions (Pekrun et al., 2006). Moreover, the mode of tillage can influence seed persistence (Gruber et al., 2010) likely due to its effects on burial depth, water supply, gaseous, light and temperature conditions.

1.2.4 Volunteer OSR

OSR volunteers can occur in the subsequent crops even more than 10 years after harvest due to long-term seed persistence (Lutman et al., 2003; Mess éan et al., 2007; Belter, 2016). These volunteers can cause problems in weed control in directly following crops such as cereals, or in another cultivated OSR some years later in the crop rotation. Weed control will become more difficult if the volunteers are herbicide tolerant. Moreover, unwanted gene pollination or seed admixture would spoil seed quality and oil quality of grown OSR if varieties with different traits such as Holli-OSR was previously grown in the same fields or in the neighbouring fields. Some large scale farm studies in France showed that seed admixture of oilseed rape > 0.9% can occur in OSR as following crops to other OSR crops even 3-8 year later (Mess éan et al., 2007).

The emergence of OSR volunteers varies considerably between varieties in time. Volunteers from varieties with high disposition to dormancy are supposed to persist for a longer period (Gulden et al., 2003; Pekrun et al., 2005; Gruber and Claupein, 2007). Seed bank size is a crucial factor to determine the amounts of volunteers, but not the only one. For instance, much more volunteers were recorded of a high dormancy variety in the first crop after OSR due to the larger soil seed bank, compared to a low dormancy variety (Gruber et al., 2004c; Weber et al., 2014). In addition, seed burial by tillage is another important influencing factor. Non-inversion tillage (i.e. chisel ploughing or rototiller) results in greater volunteers than inversion tillage (i.e., mouldboard ploughing) due to shallow seed burial in the soil (Gruber et al., 2004b, 2005); in no-till conditions, much more volunteers can be found if no effective weed control was performed. Also, soil texture likely affects occurrence of volunteers probably related to growth resistance by soil clods (Dürr et al., 2001; Sester et al., 2007) and to moisture and oxygen provided by different soils (Gruber et al., 2014).

Several approaches to the control of OSR volunteers have been proposed such as selecting or breeding for low dormancy variety, crop management (including crop rotation and tillage systems) and herbicide application. However, the available information in these aspects and the relevant mechanisms are still limiting especially from long-term experiments.

1.3 Imidazolinone-tolerant oilseed rape (Clearfield® oilseed rape; CL OSR)

Imidazolinone herbicides were discovered in the 1970s at the American Cyanamid Company (Shaner and O'Connor, 1991). To date, six imidazolinone herbicides (imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin) have been registered (Ramezani, 2007). These herbicides target the enzyme acetohydroxyacid synthase (ALS), which is the first common enzyme for the biosynthesis of branched chain amino acids in plants (Ray, 1984). A broad spectrum of weeds (both monocotyledonous and dicotyledonous) can be controlled by ALS-inhibiting herbicides at low application rates pre- or post-emergence of crop. This option could provide benefits for the cropping systems.

CL OSR was first developed in the late 1980s in Canada using microspores and haploid protoplasts (Swanson et al., 1988, 1989). By spraying imidazolinone herbicides (i.e. imazethapyr), five double-haploids were selected in the greenhouse, and two mutants (point mutations *PM1* and *PM2*) showed super tolerance to imazethapyr. The genes *PM1* and *PM2* are unlinked and additive, and they are estimated to link to the ALS1 and ALS3 loci of OSR, respectively (Rutledge et al., 1991). Regarding herbicide tolerance, *PM1* confers tolerance to imidazolinones only, but *PM2* is tolerant to both imidazolinones and sulfonylureas (Shaner et al., 1996) so the tolerance level resulting from *PM2* is much higher than that from PM_1 (Hattori et al., 1995). The highest tolerance level to imidazolinone herbicides can be achieved when both *PM1* and *PM2* are homozygous (Tan et al., 2005), which has already been realized in commercial CL OSR varieties.

Due to possible benefits for weed control by the Clearfield® production system, the combination of CL varieties and imidazolinone herbicides, CL crops such as maize (*Zea mays* L.), oilseed rape (*B. napus*), rice (*Oryza sativa*), wheat (*Triticum aestivum*), sunflower (*Helianthus annuus*) and lentil (*Lens culinaris*) have already been commercially used (Tan et al., 2005; Pfenning et al., 2008). CL OSR has already been widely grown in

the United States and Canada (Beckie et al., 2004; Brimner et al., 2005). In EU, some of CL OSR varieties have already been introduced in Eastern part of Europe and will comprehensively continue in other regions. Despite all these, the seed dormancy and potential volunteer problems resulting from CL OSR have not been deeply detected yet. The debate about CL OSR is rising in Europe and Germany due to CL OSR volunteers in other crops, which might not be controlled easily by many herbicides using sulfonylureas and other active ingredients to which a cross-tolerance exists (Krato et al., 2012). On the other hand, CL OSR volunteers in grown OSR, which cannot be killed by spraying herbicides, could outcross, admix, and multiply in the long run.

1.4 Outline and objectives

The overall objective of this thesis was to propose suitable strategies to reduce CL OSR volunteers in the subsequent crops in terms of variety choice based on seed dormancy and crop management. This resulted in the following hypotheses:

1. (i) There is primary (innate) and secondary (induced) dormancy in oilseed rape; (ii)Primary dormancy decreases during seed development, the potential secondary dormancy increases; (iii) At maturity, the level of the remaining primary dormancy and the varietal potential to secondary dormancy probably correlate.
2. (i) There is variation in potential seed dormancy of CL OSR. (ii) F_1 (seeded) and F_2 (harvested) generations of hybrid CL-OSR show similar dormancy levels although changes through environmental effects are known; (iii) the environment (location) during seed development and maturation has an effect on the potential dormancy.
3. (i) The soil seed bank size of OSR is determined by post-harvest tillage (particularly tillage time) and seed dormancy traits of the cultivated variety. (ii) The emergence of volunteers from the seed bank also depends on the mode of tillage. (iii) Gene segregation in herbicide-tolerance might occur between CL volunteers.

For the investigation of these hypotheses, seed dormancy in F_1 and F_2 generations of 15 CL OSR accessions was investigated in the laboratory. Several field experiments were conducted at different locations in south-west Germany during 2012-2016. The experimental results were shown in Chapter I- III that were published or peer-reviewed manuscripts.

Chapter I deals with the development of primary dormancy and potential secondary dormancy in OSR and their interaction during seed development. **Chapter II** focuses on potential secondary dormancy level in mature seeds of CL OSR, its inheritance from F_1 to F_2 generations, and its interaction with maternal environment. **Chapter III** examines the effects of variety, tillage (tillage mode and operation time), and their interaction on soil seed bank and volunteers in the subsequent crops after OSR.

2 Publications

The present thesis consists of three scientific articles as reflected by chapter I-III, which form the body of the dissertation. These articles have been published. For citation of the three articles, please use the references given below.

Chapter I

Huang, S., Gruber, S., Stockmann, F., Claupein, W. (2016): **Dynamics of dormancy during seed development of oilseed rape (*Brassica napus* L.).** Seed Science Research, 1, 1-9

Chapter II

Huang, S., Gruber, S., Weber, E. A., Claupein, W. **Seed dormancy in F_1 and F_2 generations of imidazolinone-tolerant oilseed rape at different locations.** Journal für Kulturpflanzen/Journal of Cultivated Plants, 68, 175-184.

Chapter III

Huang, S., Gruber, S., Claupein, W. **Field history of imidazolinone-tolerant oilseed rape (*Brassica napus*) volunteers in following crops under six long-term tillage systems.** Field Crops Research, 185, 51-58.

3 Chapter I

Dynamics of dormancy during seed development of oilseed rape (*Brassica napus* L.).

Publication I

Huang, S., Gruber, S., Stockmann, F., Claupein, W. (2016):
Dynamics of dormancy during seed development of oilseed rape (*Brassica napus* L.).
Seed Science Research

The contribution of seed dormancy, particularly secondary dormancy, to seed persistence in the soil seed bank of oilseed rape was frequently highlighted by previous studies, but the information about dormancy formation during seed development is quite limited. This chapter focused on the dynamics of primary dormancy and potential induced secondary dormancy over time during seed development of oilseed rape, providing important information for the understanding of underlying mechanisms in the formation, fluctuation, maintenance, and decline of seed dormancy in oilseed rape.

Seed Science Research (2016), page 1 of 9
doi:10.1017/S0960258516000118

Dynamics of dormancy during seed development of oilseed rape (*Brassica napus* L.)

Shoubing Huang*, Sabine Gruber, Falko Stockmann and Wilhelm Claupein
University of Hohenheim, Institute of Crop Science, Fruwirthstr. 23, 70599 Stuttgart, Germany

(Received 21 January 2016; accepted after revision 14 April 2016)

Abstract

Seed dormancy is a critical factor in determining seed persistence in the soil and can create oilseed rape (*Brassica napus* L.) volunteer problems in subsequent years. A 3-year field trial in south-west Germany investigated the effects of seed maturity on primary dormancy and disposition to secondary dormancy of ten oilseed rape varieties (lines) in 2009 and 2010, and of five imidazolinone-tolerant varieties (hybrids) in 2014. Fresh seeds were sampled weekly from about 30 d after flowering (DAF) until full maturity and tested for dormancy on the day of seed collection. Primary dormancy decreased from a high level of 70–99% at 30–40 DAF to 0–15% after 7–14 d, coinciding with embryo growth and depending on variety and year. For some oilseed rape varieties, 30–50% primary dormancy was still present in mature seeds. Depending on variety, disposition to secondary dormancy was nearly zero at the early stage of seed development, increased to its highest level during development, and decreased afterwards. Some varieties maintained a high level of secondary dormancy at maturity or during the entire seed development period. The correlation between primary dormancy and secondary dormancy was significantly positive at early seed development ($r = 0.95$, 50 DAF), but declined in mature seeds. Environmental conditions during ripening are also expected to affect dormancy dynamics. The deeper insights into dormancy formation of oilseed rape provide the possibility to improve harvest time and harvest method, and to better assess the potential for volunteer oilseed rape in following crops.

Keywords: abscisic acid, canola, dormancy, harvest date, seed bank, volunteers

* Correspondence
Email: shuang@uni-hohenheim.de

Introduction

Volunteer oilseed rape (*Brassica napus* L.) is an issue of concern due to the difficulty of chemical control of volunteers in a sown crop, especially when the varieties of oilseed rape are tolerant to specific herbicides such as imidazolinone. The emergence of volunteer oilseed rape is strongly dependent on seed persistence in the soil seed bank, which can last up to 11 years (Lutman *et al.*, 2003). Secondary dormancy, induced by external environmental conditions such as dryness, darkness or oxygen deficiency (Pekrun *et al.*, 1997, 1998) is a crucial contributor to seed persistence (Pekrun *et al.*, 1998; Gulden *et al.*, 2003, 2004a), in interaction with varieties (Gruber and Claupein, 2007a; Messéan *et al.*, 2007; Weber *et al.*, 2014). Gruber *et al.* (2010) found that 60% of initially buried seeds of a high-dormancy variety were still viable after 4.5 years of burial in the soil, while only 8% of a low-dormancy variety survived. Accordingly, a 30-fold increase in volunteer oilseed rape was recorded after growing and harvesting a high-dormancy variety compared to a low-dormancy variety in spring of the first following crop of winter wheat (Weber *et al.*, 2014).

In contrast to secondary dormancy, primary dormancy is expressed in underdeveloped embryos and is controlled by phytohormones (mainly abscisic acid, ABA) (Hilhorst, 1995; Baskin and Baskin, 2004), preventing pre-harvest sprouting on the mother plant. Primary dormancy has previously been shown to be low (Momoh *et al.*, 2002; Gruber *et al.*, 2004; Gulden *et al.*, 2004a) or nearly absent in fully mature seeds of oilseed rape (Schlink, 1993). Evidence has been found that primary dormancy may sometimes be present at harvest but that it decreases with the duration of dry storage (Gruber *et al.*, 2004; Haile and Shirtliffe, 2014).

Dormancy dynamics are probably linked to a set of environmental conditions (e.g. precipitation or temperature) during the crop growth period. There appears to be a link between secondary dormancy and year (Gruber *et al.*, 2009). For example, environmental conditions generally considered 'optimal' for seed yield have been shown to result in comparatively high levels of dormancy in winter oilseed rape

(Schatzki *et al.*, 2013a), and Gulden *et al.* (2004a) showed that the environment contributed 0.1–4.5% to secondary dormancy in spring oilseed rape.

Neither the degree of primary dormancy of oilseed rape nor how long it is able to express secondary dormancy before harvest is well understood. This information could be especially relevant for oilseed rape fields that have been damaged by hailstorms in the weeks before harvest, to assess the potential of their seeds both to fall dormant and to result in volunteers in following crops. In two studied oilseed rape varieties, primary dormancy decreased over time during seed development, while secondary dormancy tended to increase (Haile and Shirtliffe, 2014). In fully mature oilseed rape seeds, there is a weak positive correlation between primary dormancy and secondary dormancy (Schatzki *et al.*, 2013b) but this has not been observed with respect to different stages of seed development. Whether there is an interaction between primary and secondary dormancy in oilseed rape and, if there is, how this interaction occurs during seed development are important for understanding seed dormancy in oilseed rape. If a correlation between them could be demonstrated, prediction of secondary dormancy by the level of primary dormancy would be possible. The objectives of this study were to determine the development of primary dormancy and secondary dormancy in oilseed rape, and, further, to find correlations between these during seed development.

Materials and methods

Field trials

Field trials were conducted in the years 2009, 2010 and 2014 at the experimental station Ihinger Hof (IHO) of the University of Hohenheim, south-west Germany (48°45′N, 8°56′E) on a loam soil. Mean annual temperature is 7.9°C and mean precipitation is 690 mm. Mean daily temperatures from flowering to harvest of oilseed rape (May–July) in 2009, 2010 and 2014 were 16.1, 15.8 and 15.7°C, respectively. Cumulative precipitation during this period in the 3 years was 362, 275 and 255 mm. Weather details are provided in supplementary Fig. S1. Daily temperature was calculated based on the mean hourly air temperature at 2 m above soil, and cumulative precipitation was derived from the daily rainfall. Weather data were obtained from the experimental station Ihinger Hof.

The trials were arranged in a randomized block design with four parallel blocks including ten line varieties of winter oilseed rape in 2009 and 2010 and five hybrid imidazolinone-tolerant (IT) oilseed rape varieties in 2014 per block. There were four field replicates per variety with a plot size of 4 m × 8 m in all 3 years. Seeds were sown on 27 August 2008, 29 August 2009 and 21 August 2013, with a sowing density of 50 seeds m^{-2}. Pest control and fertilization were performed according to the best management practice at that location.

Flowers in full bloom of the main inflorescence of 50 randomly chosen individual plants per variety and replicate were marked in 2009 and 2010, and 30 plants per variety and replicate in 2014, in the first week of May. The label was placed within the inflorescence, below the cohort of recently opened blossoms, to indicate the flowering time of these individual flowers. About 1 month after flowering, the first five pods of the main inflorescences directly above the label of five marked plants per harvest date were picked by hand at weekly intervals in all years (supplementary Table S1). In 2009 (eight harvest timings) and 2010 (nine harvest timings), five varieties (Smart, Express, Compakt, Splendor and Nemax) were harvested first, and the remaining five varieties (Lilian, Charly, Beauty, Ladoga and Komando) were harvested 2 d later, due to different flowering stages between two variety groups; ten varieties were harvested at the final timing in 2010. In 2014 (six harvest timings), all five varieties were harvested on the same day at each harvest.

Assessment of primary and secondary dormancy

The seeds of the five pods were removed from the siliques, pooled and then tested for primary dormancy and secondary dormancy according to the Hohenheim Standard Dormancy Test (Weber *et al.*, 2010) on the same day of seed collection. The test of secondary dormancy is comprised of three sequential processes. First, dormancy induction: 100 seeds per variety and harvest date in four laboratory replicates were imbibed in 8 ml of a polyethylene glycol 6000 solution (354.4 g l^{-1}, −15 bar; Michel and Kaufmann, 1973) in 9-cm plastic Petri dishes with double layers of filter paper. The Petri dishes were then put in metal boxes lined with black film to exclude any light and water evaporation, and were transferred to an incubator (20°C) for 14 d. Second, germination of non-dormant seeds: seeds were transferred to new Petri dishes equipped with double layers of filter paper and 6 ml deionized water in darkness at 20°C (14 d). During this period, seeds that were not induced into dormancy germinated and were removed from Petri dishes under a green safety light (500–600 nm). Third, dormancy breaking: non-germinated seeds were subjected to alternating light and temperature (12 h, 3°C, darkness/12 h, 30°C, light) for 7 d; germinated seeds in this process were considered to be induced into secondary dormancy in the first process. Seeds that germinated in the above three processes were considered viable. Intact seeds that did not germinate after dormancy breaking were

considered dead or unable to germinate due to any other reasons; usually none or very few of these seeds existed. Secondary dormancy level was the proportion of induced dormant seeds over the total number of viable seeds used in the assays.

A parallel test for primary dormancy included the same two latter processes, germination of non-dormant seeds in darkness and dormancy breaking, but excluded a preceding dormancy induction in polyethylene glycol solution. Seeds that did not germinate before dormancy breaking were declared primarily dormant. Primary dormancy level was the proportion of dormant seeds over the total number of all viable seeds found in a sample. Seed viability was the proportion of the sum of any germinated seeds in a sample (during germination test and dormancy breaking) to all seeds used in the sample (Gruber *et al.*, 2004; Weber *et al.*, 2010).

Secondary dormancy in the fourth to ninth harvests in 2010 and in all harvests in 2014 was tested; primary dormancy was tested for seeds harvested at each harvest in all 3 years. Before starting the tests, broken or obviously mouldy seeds were excluded from seed lots.

Seed water content

Seed water content, as a measure of the developmental stage and grade of maturity of the seeds, was determined as follows: a portion of the harvested seed lot was dried in a 60°C oven for 48 h and seed water content was calculated as the per cent water content in the fresh seed weight. Additionally, embryos were removed from the seeds from another part of the harvested seed lot and photographed to document the stage of seed development.

Statistical analysis

Dormancy values were arc-sin transformed to stabilize variance and adjust the data to a standard normal distribution, with the following formula (Chatterjee and Hadi, 2012):

$$y = \arcsin\left(\sqrt{\frac{d+3/8}{v+3/4}}\right);$$

where y is the transformed value, d the number of dormant seeds, and v the number of viable seeds per replicate. The adjusted data were back-transformed for presentation. Analyses of variance for primary and secondary dormancy were performed according to the experimental design using the PROC MIXED procedure of the statistical software SAS 9.3 (SAS Institute, Cary, North Carolina, USA). All main effects for varieties, harvest date and their interactions were taken as fixed, while effects for laboratory replicates were taken as random. The correlations between primary dormancy and secondary dormancy at different harvests were analysed with the procedure PROC CORR of SAS 9.3.

Results

The water content of oilseed rape seeds harvested at different dates decreased continuously from approximately 80% at 28 d after flowering (DAF) to 9% when seeds were fully mature in 2009 and 2010 (Fig. 1). The decrease in seed water content ranged from 40% to 4% during the monitoring period of 2014. In 2009 and 2010, seed viability increased rapidly from nearly zero (28 DAF) to 100% (49 DAF) within 3 weeks, depending on variety, and remained at 100% until final harvest (Fig. 2). In 2014, seed viability was

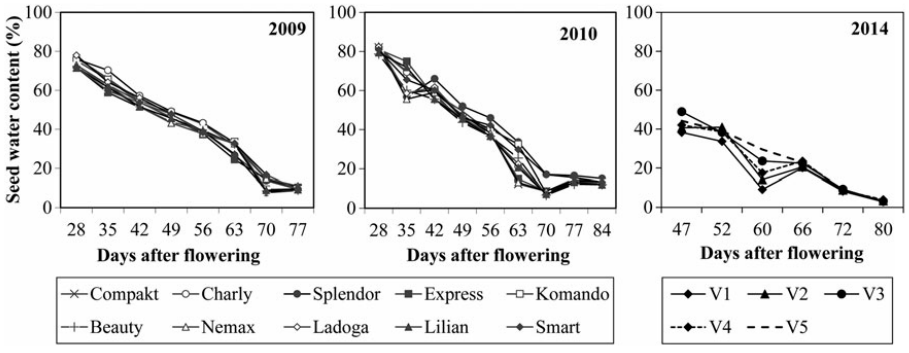

Figure 1. Seed water content (%) during seed development of ten oilseed rape varieties (lines) in 2009 and 2010, and of five hybrid imidazolinone-tolerant oilseed rape varieties (V1–V5) in 2014; for harvest dates see supplementary Table S1.

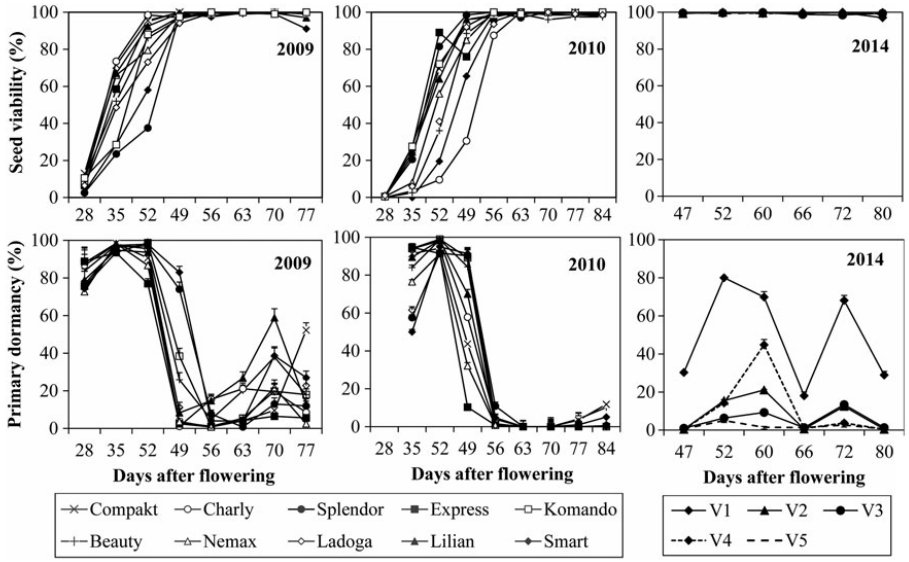

Figure 2. Seed viability (%) and primary seed dormancy (%) during seed development of ten oilseed rape varieties (lines) in 2009 and 2010, and of five hybrid imidazolinone-tolerant oilseed rape varieties (V1–V5) in 2014; for harvest dates see supplementary Table S1; error bars indicate standard error of mean.

already 100% at the first harvest, and lasted until the final harvest. Embryo development was exemplarily documented by images taken of the variety Smart (supplementary Fig. S2); the embryo was green at the beginning of the study, and over time it lost chlorophyll and turned yellow.

Variety, harvest date and their interaction had significant effects on both primary dormancy and secondary dormancy of oilseed rape (Table 1). Primary dormancy at the first harvest (28 DAF) in 2010 was not shown because of low seed viability (0.4%; Fig. 2). The primary dormancy values in viable seeds of ten line varieties were 73–89% at 28 DAF in 2009 and 50–95% at 35 DAF in 2010, depending on variety, peaking at 100% at 42 DAF, and decreased to zero in another 7–14 d (Fig. 2). Primary dormancy in varieties Compakt, Smart and Ladoga was still at 20–50% at the last harvest (*c.* 80 DAF) in 2009, but decreased to below 20% in 2010, similar to the other varieties. When seeds were fully viable (47 DAF) in 2014, primary dormancy was nearly zero for varieties V2–V5, was 30% for V1, and increased to the highest level at 52 or 60 DAF,

Table 1. Analysis of variance for primary dormancy and secondary dormancy of oilseed rape in 2009, 2010 and 2014, as affected by variety (V), harvest date (D) and their interactions (V × D)

Effect	DF	2009[a]		DF	2010[b]		DF	2014[c]	
		F value	*P*		*F* value	*P*		*F* value	*P*
Primary dormancy									
Variety (V)	9	15.9	<0.0001	9	9.7	<0.0001	4	609.8	<0.0001
Date (D)	7	1493.6	<0.0001	7	2537.8	<0.0001	5	210.0	<0.0001
V × D	63	16.1	<0.0001	63	13.6	<0.0001	20	21.3	<0.0001
Secondary dormancy									
Variety (V)	–	–	–	9	90.0	<0.0001	4	521.7	<0.0001
Date (D)	–	–	–	5	300.0	<0.0001	5	79.3	<0.0001
V × D	–	–	–	45	15.4	<0.0001	20	9.9	<0.0001

[a]Ten line varieties of winter oilseed rape, eight harvest dates; not analysed: secondary dormancy in 2009.
[b]Ten line varieties of winter oilseed rape, nine harvest dates; not analysed: primary dormancy at the first harvest because of low seed viability, secondary dormancy from the first harvest to the third harvest.
[c]Five hybrid imidazolinone-tolerant oilseed rape varieties, six harvest dates.

followed by a non-linear decrease with time. At the final harvest in 2014, primary dormancy of varieties V2–V5 were nearly zero, but variety V1 still had 29% primary dormancy.

Secondary dormancy was initially low at 50–60 DAF (2010), reached a peak at about 70 DAF, depending on variety, and gradually decreased over time (Fig. 3). However, there were still four varieties that showed quite high secondary dormancy (>40%) at the last harvest in 2010. Particularly, secondary dormancy in variety Smart continued to increase from 31% at 49 DAF to 62% at 84 DAF, the highest dormancy among ten varieties at the final harvest. The levels of secondary dormancy tended to remain stable or to decrease slightly in the varieties V1, V2, V3 and V4 in the 2014 trial; V5 showed a clear decrease in secondary dormancy during that time.

As seed development progressed, the percentage values of both primary and secondary dormancy did not closely correlate with seed water content (data not shown), or with morphological seed development in general.

A significant correlation between primary dormancy and secondary dormancy was only found in the early stage of seed development (Table 2); correlation coefficients decreased from 0.58 at about 50 DAF to 0.09 at the final harvest in 2010, and deceased from 0.95 at 47 DAF to 0.69 at the final harvest in 2014. Both primary dormancy and secondary dormancy were not significantly correlated with cumulative precipitation and temperature during the harvest intervals (data not shown).

Discussion

This study indicates that primary dormancy decreased and the disposition to secondary dormancy (induced

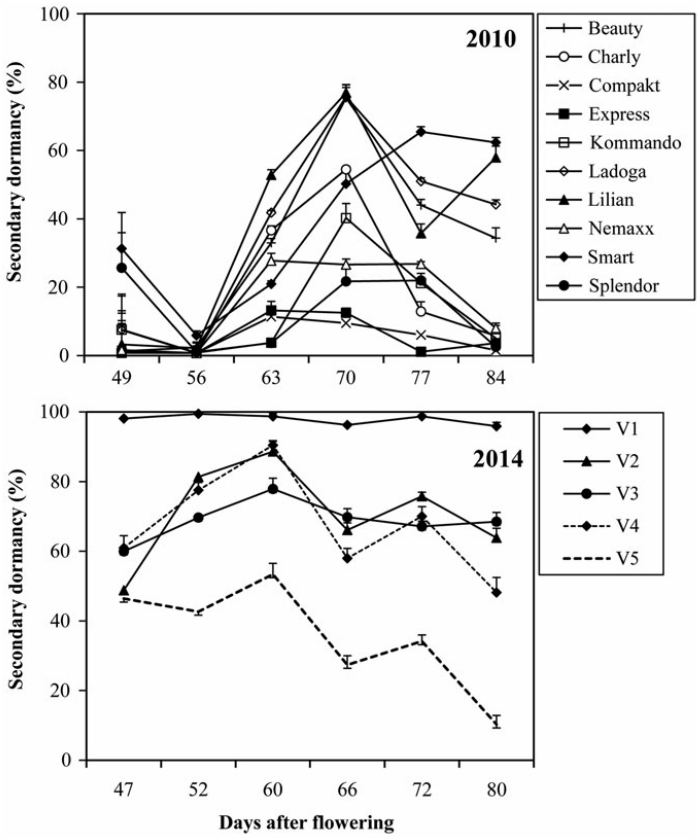

Figure 3. Dynamics of secondary dormancy (%) during seed development of ten oilseed rape varieties (lines) in 2010, and of five hybrid imidazolinone-tolerant oilseed rape varieties (V1–V5) in 2014; for harvest dates see supplementary Table S1; error bars indicate standard error of mean.

Table 2. Correlation coefficients (r) between primary dormancy and secondary dormancy at different harvest dates during seed development of oilseed rape in the years 2010 ($n = 10$) and 2014 ($n = 5$); experimental station Ihinger Hof, south-west Germany; ten line varieties of winter oilseed rape in 2010 and five hybrid imidazolinone-tolerant oilseed rape varieties in 2014. DAF, days after flowering. For harvest dates see supplementary Table S1

Year	Correlation coefficient (r)					
2010	49 DAF	56 DAF	63 DAF	70 DAF	77 DAF	84 DAF
($n = 10$)	0.58*	0.18 ns	0.00 ns	0.00 ns	0.26 ns	0.09 ns
2014	47 DAF	52 DAF	60 DAF	66 DAF	72 DAF	80 DAF
($n = 5$)	0.95*	0.77	0.80 ns	0.73 ns	0.78 ns	0.69 ns

*$P < 0.05$; ns, not significant.

dormancy) of oilseed rape increased during the two months before typical harvest (normally at the end of July at the experimental site), in agreement with the findings of Gulden *et al.* (2004a), Gruber and Claupein (2007b), and Haile and Shirtliffe (2014). However, a rapid decline in primary dormancy in as short a period as 1 week, as seen in the present study (42–49 DAF in 2009, and 49–56 DAF 2010; Fig. 2) – coinciding with a rapid increase in seed viability and with embryo colour change from green (49 DAF) to yellow (56 DAF; supplementary Fig. S2) – had not been demonstrated to date. This rapid reduction in primary dormancy was not observed in 2014, probably due to the observed high seed viability of about 100% at first harvest (47 DAF). The rapid increase of seed viability at early seed development is supposed to depend on a critical growing period of embryos, after which almost all the seeds have a capacity to germinate if primary dormancy can be broken (Holdsworth *et al.*, 1999, 2001). Based on the calculation of seed dormancy in the present study, all the dormant seeds were viable. Therefore, the observed high primary dormancy before the rapid decline at early seed development may be attributable to development of the embryos (Bewley, 1997; Haile and Shirtliffe, 2014). This process is assumed to depend on variety: primary dormancy in six out of ten varieties decreased to below 10% at 49 DAF, and the other four varieties (Smart, Splendor, Komado and Beauty) needed another 7 d (56 DAF) to reach the same dormancy level (in 2009). Using the water content of the seeds as an indicator of seed development, there was no apparent difference in water decrease between the varieties, so this would not explain differences in dormancy. Thus, the levels and changes in dormancy do not seem to be linked directly to morphological and physiological ripening alone. However, there seems to be a critical stage of development at which primary dormancy starts to decline. This stage may depend somehow on embryo development.

Various studies have documented the regulation of onset and maintenance of primary dormancy in many crop species by ABA (Finkelstein *et al.*, 1985; Bewley, 1997; Gubler *et al.*, 2005; Nambara *et al.*, 2010). ABA content accumulated during seed development in oilseed rape, and reached its highest level at 35–40 DAF (Finkelstein *et al.*, 1985), coinciding with the second to third harvests in 2009 and 2010 in our study, then declined with seed development. The appearance of ABA peaks during seed development can be influenced by environmental conditions, such as rainfall, temperature and humidity, surrounding mother plants (King, 1993; Romagosa *et al.*, 2001; Chono *et al.*, 2006). This may explain observed differences in the onset of the decline of primary dormancy in seed development between years.

Dormancy variation, particularly for primary dormancy, between varieties in the late seed development might be attributed to the temporal effects of environmental conditions (e.g. precipitation). However, correlations of primary and secondary dormancy with cumulative temperature and precipitation the week before harvest were not significant (not shown). Harvest timing experiments conducted at different locations or under controlled conditions could probably offer more robust data for detecting this relationship.

Secondary dormancy of most varieties used in the current study increased after the immature seeds achieved full viability, consistent with the findings of Haile and Shirtliffe (2014), then decreased to different degrees (Fig. 3). In oilseed rape, there is consensus that secondary dormancy decreases with storage time post-harvest (Gulden *et al.*, 2004a), which is likely to be connected with the decline of secondary dormancy when seeds are still on the mother plants. With this in mind, this dormancy decline might be a continuous process in time, which starts before seed maturity and continues during seed storage. Also, the potential dynamics of secondary dormancy are dependent on the variety, as shown in Table 1 and Fig. 3. The importance of variety has been reported previously in European, Canadian and Chinese oilseed rape (Momoh *et al.*, 2002; Gruber *et al.*, 2004, 2009; Gulden *et al.*, 2004a; Schatzki *et al.*, 2013a, b), but these studies focused mainly on the mature seeds. The present study indicates that genotypic differences in secondary dormancy are initially small, but become greater with seed development. This result may provide some

clues to understanding the formation and maintenance of secondary dormancy, if differences in genetic background between varieties can be detected clearly enough.

Similar to primary dormancy, secondary dormancy in oilseed rape is also presumed to be associated with ABA (Gulden *et al.*, 2004b; Fei *et al.*, 2007, 2009). More ABA-inducible genes were up-regulated in mature seeds of a high-dormancy oilseed rape variety compared to a low-dormancy variety when treated with polyethylene glycol; twice as much ABA was detected in freshly harvested seeds of a high-dormancy variety (Fei *et al.*, 2007, 2009) and high-dormancy oilseed rape varieties seem to be more sensitive to ABA than low-dormancy varieties (Gulden *et al.*, 2004b). Despite the complex mechanisms involved in the dynamics of secondary dormancy, which are not yet completely understood, the results of this study indicate that harvest before full seed maturity might lead to high dormancy levels (mainly secondary dormancy) in the seeds, but accompanied by low harvest losses (Price *et al.*, 1996). Also, there seems to be a certain degree of primary dormancy in mature seeds, which could lead to a soil seed bank even if there are no further dormancy-inducing conditions. In contrast, late harvest can result in low-dormancy seeds, but the harvest losses will be great (e.g. *c.* 5000 seeds m^{-2}; Lutman *et al.*, 2005). With the improvement of harvesting techniques and breeding (pod-shattering resistance), the latter option could become a more acceptable way to reduce volunteer problems in a crop rotation with oilseed rape. However, a problem would arise if, for example, hailstorms cracked unripe pods and fresh seeds dropped (e.g. more than 280 hailstorms were recorded in June and July 2014 across Germany; ESWD, 2014). These fresh seeds with high levels of primary dormancy could lead to seed survival in the soil without further inductive environmental conditions, and could be induced into secondary dormancy by environmental conditions such as dryness (Pekrun *et al.*, 1997, 1998). Based on our results, seed dormancy (primary and secondary dormancy) is variety-dependent during late seed development; i.e. varieties with low dormancy represent a lower risk of a soil seed bank after hailstorms or after early harvest.

A significant correlation between primary dormancy and secondary dormancy at early stages of seed development in oilseed rape, as shown in the current study, could be used to predict roughly the level of secondary dormancy from a specific stage of development. Because of environmental effects, however, this prediction would be most useful for plants grown under controlled conditions, such as in a greenhouse. In a different plant, *Arabidopsis thaliana*, it was shown that the state of primary seed dormancy can affect secondary dormancy induction after harvest (Auge *et al.*, 2015). In this case, secondary dormancy was more easily induced in seeds with higher primary dormancy than in seeds with low or no primary dormancy of the same *A. thaliana* variety that matured under a different maternal environment. This may also occur in oilseed rape during late seed development, based on the positive correlation between primary dormancy and secondary dormancy, but depends on the year and the variety.

Because both primary and secondary dormancy are present in immature seeds of oilseed rape, both timing and techniques of harvest should be reconsidered. Early harvest, for example windrowing before full maturity, may reduce harvest losses caused by pod shattering, but could lead to higher seed dormancy. Afterripening, which was not tested in this study, would further decrease dormancy. However, in the context of increasingly worldwide use of the combine harvester, harvest at full maturity or late harvest is preferred but could cause large harvest losses. Under this harvest scenario, selection of low-dormant oilseed rape varieties would be a simple and feasible strategy to reduce the risk of soil seed bank and volunteers in a crop rotation.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0960258516000118

Acknowledgements

The authors would like to thank Andrea Rückle for excellent technical support, the staff of the experimental station Ihinger Hof for excellent field management, Dr Sebastian Munz, Jialu Xu and Kathleen Regan for their useful comments, and the China Scholarship Council (CSC) for their support.

Financial support

Financial support was provided by the German Federal Ministry of Education and Research (BMBF 0315211A) and China Scholarship Council (CSC 201206350064).

Conflicts of interest

None.

References

Auge, G.A., Blair, L.K., Burghardt, L.T., Coughlan, J., Edwards, B., Leverett, L.D. and Donohue, K. (2015)

Secondary dormancy dynamics depends on primary dormancy status in *Arabidopsis thaliana*. *Seed Science Research* **25**, 1–17.

Baskin, J.M. and Baskin, C.C. (2004) A classification system for seed dormancy. *Seed Science Research* **14**, 1–16.

Bewley, J.D. (1997) Seed germination and dormancy. *Plant Cell* **9**, 1055–1066.

Chatterjee, S. and Hadi, A.S. (2012) *Regression analysis by example* (5th edition). Hoboken, NJ, USA, John Wiley & Sons.

Chono, M., Honda, I., Shinoda, S., Kushiro, T., Kamiya, Y., Nambara, E., Kawakami, N., Kaneko, S. and Watanabe, Y. (2006) Field studies on the regulation of abscisic acid content and germinability during grain development of barley: molecular and chemical analysis of pre-harvest sprouting. *Journal of Experimental Botany* **57**, 2421–2434.

ESWD (European Severe Weather Database). (2014) Available at http://www.eswd.eu/cgi-bin/eswd.cgi (accessed April 2016).

Fei, H., Tsang, E. and Cutler, A.J. (2007) Gene expression during seed maturation in *Brassica napus* in relation to the induction of secondary dormancy. *Genomics* **89**, 419–428.

Fei, H., Ferhatoglu, Y., Tsang, E., Huang, D. and Cutler, A.J. (2009) Metabolic and hormonal processes associated with the induction of secondary dormancy in *Brassica napus* seeds. *Botany* **87**, 585–596.

Finkelstein, R.R., Tenbarge, K.M., Shumway, J.E. and Crouch, M.L. (1985) Role of ABA in maturation of rapeseed embryos. *Plant Physiology* **78**, 630–636.

Gruber, S. and Claupein, W. (2007a) Primäre Dormanz in reifenden Rapssamen. *Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften* **19**, 34–35.

Gruber, S. and Claupein, W. (2007b) Fecundity of volunteer oilseed rape and estimation of potential gene dispersal by a practice-related model. *Agriculture, Ecosystems & Environment* **119**, 401–408.

Gruber, S., Pekrun, C. and Claupein, W. (2004) Seed persistence of oilseed rape (*Brassica napus*): variation in transgenic and conventionally bred cultivars. *Journal of Agricultural Science* **142**, 29–40.

Gruber, S., Emrich, K. and Claupein, W. (2009) Classification of canola (*Brassica napus*) winter cultivars by secondary dormancy. *Canadian Journal of Plant Science* **89**, 613–619.

Gruber, S., Bühler, A., Möhring, J. and Claupein, W. (2010) Sleepers in the soil – vertical distribution by tillage and long-term survival of oilseed rape seeds compared with plastic pellets. *European Journal of Agronomy* **33**, 81–88.

Gubler, F., Millar, A.A. and Jacobsen, J.V. (2005) Dormancy release, ABA and pre-harvest sprouting. *Current Opinion in Plant Biology* **8**, 183–187.

Gulden, R.H., Shirtliffe, S.J. and Thomas, A.G. (2003) Secondary seed dormancy prolongs persistence of volunteer canola in western Canada. *Weed Science* **51**, 904–913.

Gulden, R.H., Thomas, A.G. and Shirtliffe, S.J. (2004a) Relative contribution of genotype, seed size and environment to secondary seed dormancy potential in Canadian spring oilseed rape (*Brassica napus*). *Weed Research* **44**, 97–106.

Gulden, R.H., Thomas, A.G. and Shirtliffe, S.J. (2004b) Secondary dormancy, temperature, and burial depth regulate seedbank dynamics in canola. *Weed Science* **52**, 382–388.

Haile, T.A. and Shirtliffe, S.J. (2014) Effect of harvest timing on dormancy induction in canola seeds. *Weed Science* **62**, 548–554.

Hilhorst, H.W.M. (1995) A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research* **5**, 61–73.

Holdsworth, M., Kurup, S. and McKibbin, R. (1999) Molecular and genetic mechanisms regulating the transition from embryo development to germination. *Trends in Plant Science* **4**, 275–280.

Holdsworth, M., Lenton, J., Flintham, J., Gale, M., Kurup, S., McKibbin, R., Bailey, P., Larner, V. and Russell, L. (2001) Genetic control mechanisms regulating the initiation of germination. *Journal of Plant Physiology* **158**, 439–445.

King, R.W. (1993) Manipulation of grain dormancy in wheat. *Journal of Experimental Botany* **44**, 1059–1066.

Lutman, P.J.W., Freeman, S.E. and Pekrun, C. (2003) The long-term persistence of seeds of oilseed rape (*Brassica napus*) in arable fields. *Journal of Agricultural Science* **141**, 231–240.

Lutman, P.J., Berry, K., Payne, R.W., Simpson, E., Sweet, J. B., Champion, G.T., May, M.J., Wightman, P., Walker, K. and Lainsbury, M. (2005) Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (*Brassica napus*). *Proceedings of the Royal Society of London B: Biological Sciences* **272**, 1909–1915.

Messéan, A., Sausse, C., Gasquez, J. and Darmency, H. (2007) Occurrence of genetically modified oilseed rape seeds in the harvest of subsequent conventional oilseed rape over time. *European Journal of Agronomy* **27**, 115–122.

Michel, B.E. and Kaufmann, M.R. (1973) The osmotic potential of polyethylene glycol 6000. *Plant Physiology* **51**, 914–916.

Momoh, E.J.J., Zhou, W.J. and Kristiansson, B. (2002) Variation in the development of secondary dormancy in oilseed rape genotypes under conditions of stress. *Weed Research* **42**, 446–455.

Nambara, E., Okamoto, M., Tatematsu, K., Yano, R., Seo, M. and Kamiya, Y. (2010) Abscisic acid and the control of seed dormancy and germination. *Seed Science Research* **20**, 55–67.

Pekrun, C., Lutman, P.J.W. and Baeumer, K. (1997) Induction of secondary dormancy in rape seeds (*Brassica napus* L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. *European Journal of Agronomy* **6**, 245–255.

Pekrun, C., Hewitt, J.D.J. and Lutman, P.J.W. (1998) Cultural control of volunteer oilseed rape (*Brassica napus*). *Journal of Agricultural Science* **130**, 155–163.

Price, J.S., Hobson, R.N., Neale, M.A. and Bruce, D.M. (1996) Seed losses in commercial harvesting of oilseed rape. *Journal of Agricultural Engineering Research* **65**, 183–191.

Romagosa, I., Prada, D., Moralejo, M.A., Sopena, A., Muñoz, P., Casas, A.M., Swanston, J.S. and Molina-Cano, J.L. (2001) Dormancy, ABA content and sensitivity of a barley mutant to ABA application during seed development and after ripening. *Journal of Experimental Botany* **52**, 1499–1506.

Schatzki, J., Allam, M., Kloeppel, C., Nagel, M., Börner, A. and Möllers, C. (2013a) Genetic variation for secondary seed dormancy and seed longevity in a set of black-seeded European winter oilseed rape cultivars. *Plant Breeding* **132**, 174–179.

Schatzki, J., Schoo, B., Ecke, W., Herrfurth, C., Feussner, I., Becker, H.C. and Möllers, C. (2013b) Mapping of QTL for seed dormancy in a winter oilseed rape doubled

haploid population. *Theoretical and Applied Genetics* **126**, 2405–2415.

Schlink, S. (1993) Primäre Dormanz bei Körnerrapssorten. *Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften* **6**, 153–156.

Weber, E.A., Frick, K., Gruber, S. and Claupein, W. (2010) Research and development towards a laboratory method for testing the genotypic predisposition of oilseed rape (*Brassica napus* L.) to secondary dormancy. *Seed Science and Technology* **38**, 298–310.

Weber, E.A., Gruber, S. and Claupein, W. (2014) Emergence and performance of volunteer oilseed rape (*Brassica napus*) in different crops. *European Journal of Agronomy* **60**, 33–40.

4 Chapter II

Seed dormancy in F_1 and F_2 generations of imidazolinone-tolerant oilseed rape at different locations.

Publication II

Huang, S., Gruber, S., Weber, E. A., Claupein, W. (2016):
Seed dormancy in F_1 and F_2 generations of imidazolinone-tolerant oilseed rape at different locations.
Journal für Kulturpflanzen/Journal of Cultivated Plants.

The introduction of imidazolinone-tolerant oilseed rape into Europe increased the scepticism on the control of oilseed rape volunteers due to their tolerance to acetolactate synthase inhibiting herbicides. Seed dormancy traits of imidazolinone-tolerant oilseed rape are still not known. Besides, effects of maternal environmental on seed dormancy of oilseed rape are still not fully understood. This chapter found out that potential secondary dormancy of 15 imidazolinone-tolerant oilseed rape (Clearfield® oilseed rape) genotypes varied considerably, similar to that of conventional oilseed rape. Maternal environment during seed development could affect seed dormancy level to some extent. Particularly, precipitation might be one factor to determine seed dormancy dynamics of oilseed rape.

Chapter II: Seed dormancy in F_1 and F_2 generations of imidazolinone-tolerant oilseed rape at different locations.

Journal für Kulturpflanzen, 68 (6). S. 175–184, 2016, ISSN 1867-0911, DOI: 10.5073/JfK.2016.06.03 Verlag Eugen Ulmer KG, Stuttgart

Originalarbeit

Shoubing Huang, Sabine Gruber, Ernst Albrecht Weber, Wilhelm Claupein

Seed dormancy in F_1 and F_2 generations of imidazolinone-tolerant oilseed rape at different locations

Dormanz in Samen der F_1- und F_2- Generation von Imidazolinon-tolerantem Raps an unterschiedlichen Standorten

Abstract

The introduction of imidazolinone-tolerant oilseed rape (*Brassica napus*; Clearfield®, CL OSR) meets with skepticism on volunteer control. This study examined the disposition to secondary seed dormancy of 15 CL OSR genotypes at two locations in south-west Germany in 2012/2013 (trial 1) between sown seed (F_1) and harvested seed (F_2), and effects of maternal environment on dormancy disposition on CL and non-CL OSR in 12 locations in Germany in 2011/2012 (trial 2). The CL genotypes differed in dormancy from 0 to 95.7% in the F_1 generation and from 3.5 to 77.9% for their corresponding offspring (F_2). The dormancy levels of the F_1 generations corresponded to that of the F_2 generations. This correlation was higher if seeds derived from flowers which have been isolated in the plastic bags and thus outcrossing has been prevented. Seed lots from individual isolated F_1 plants deviated in dormancy by up to 30% from the mean of all isolated plants of a specific genotype. In trial 2, seeds from low dormancy genotypes tended to respond more strongly to maternal environment than high dormancy genotypes did. Precipitation during the period of ripening was positively correlated with dormancy (R = 0.78). Overall, breeders can use the dormancy values of the F_1 generation to assess the potential of dormancy in their offspring, which are those seeds that are relevant for causing volunteers if several other external conditions are fitting.

Key words: Clearfield, *Brassica napus*, volunteers, secondary dormancy, maternal environment, precipitation, hybrids

Zusammenfassung

Die Einführung von Imidazolinon-tolerantem Raps (*Brassica napus*; Clearfieldraps, CL Raps) wird speziell im Bereich der Kontrolle von Durchwuchsraps mit Skepsis aufgenommen. Die vorliegende Studie untersuchte die Neigung zu sekundärer Dormanz bei 15 CL Rapsgenotypen an zwei Standorten in Deutschland im Jahr 2012/2013 (Versuch 1) sowie Auswirkungen der maternalen Umgebung auf die Dormanzneigung der gebildeten Rapssamen in CL und nicht-CL Raps (insgesamt 8 Sorten) an 12 Standorten in Deutschland in den Jahren 2011/2012 (Versuch 2). Die CL-Genotypen variierten in der Dormanzneigung von 0 bis 95,7% in der F_1-Generation (Hybridsaatgut) und von 3,5 bis 77,9% in der entsprechenden F_2-Generation (Erntegut). Das Niveau der Dormanz in der F_2 entsprach dem in der F_1, sowohl bei isolierten als auch in etwas geringerem Maß bei nicht-isolierten Pflanzen. Bei allen geprüften Genotypen setzten sich die angebauten F_1-Pflanzen aus Einzelpflanzen mit zum Teil unterschiedlicher Dormanzneigung in der jeweiligen Nachkommenschaft zusammen; zum Teil wich die Dormanz der F_2 einer Einzelpflanze bis zu 30% vom Sortenmittel ab.

Institute
University of Hohenheim, Institute of Crop Science (340a), Fruwirthstr. 23, 70599 Stuttgart, Germany

Correspondence
Shoubing Huang, University of Hohenheim, Institute of Crop Science (340a), Fruwirthstr. 23, 70599 Stuttgart, Germany,
E-Mail: shoubing_huang@uni-hohenheim.de, Sabine.Gruber@uni-hohenheim.de

Accepted
30 March 2016

Chapter II: Seed dormancy in F_1 and F_2 generations of imidazolinone-tolerant oilseed rape at different locations.

Unterschiede in der Dormanz zeigten sich in zwei CL Raps-Genotypen und sechs nicht-CL-Genotypen an 12 Standorten (trial 2/Versuch 2). Samen von niedrig dormanten Genotypen reagierten tendenziell stärker auf die maternale Umgebung als Samen von hoch dormanten Genotypen. Niederschläge während der Reifezeit korrelierten positiv mit der Dormanz (R = 0,78). Insgesamt können Züchter die Dormanzwerte der F_1-Generation nutzen, um das Potential der Dormanz jener Samen in der F_2-Generation zu bewerten, die zu Durchwuchs führen könnten.

Stichwörter: Clearfield, *Brassica napus*, Durchwuchs, sekundäre Dormanz, maternale Umgebung, Niederschlag, Hybride

Introduction

The potential for gene dispersal of oilseed rape (*Brassica napus* L.; OSR) by seed, and thus by volunteers emerging from the soil seed bank, is an issue of discussion especially for herbicide tolerant OSR. Volunteer OSR is a result of high seed loss before and during harvest (LUTMAN et al., 2003, 2005), soil tillage operations after harvest, and the capacity of the seed to fall dormant (GULDEN et al., 2003; GRUBER et al., 2004a; WEBER et al., 2013). Volunteers can emerge in following crops within crop rotations grown in the same field for several years due to long-term seed persistence in the soil seed bank of up to 10 or more years (LUTMAN et al., 2005; MESSÉAN et al., 2007; D'HERTEFELDT et al., 2008). The forthcoming introduction of imidazolinone-tolerant oilseed rape (Clearfield®; CL OSR) revived the discussion about volunteers particularly because these volunteers are able to survive herbicide applications from the imidazolinone group, as well as related groups which inhibit acetolactate synthase (ALS). As chemical control of CL volunteers has to rely on a limited number of active ingredients and because chemical control of OSR volunteers in sown OSR is not possible, further agronomical strategies have to be developed and to be assessed to minimize volunteers.

Seed persistence of OSR is associated with the disposition of seeds to secondary dormancy. Soil seed banks of OSR from high dormant varieties were clearly larger than seed banks from low dormant varieties, as observed in Canada and Germany (GULDEN et al., 2003; WEBER et al., 2014). OSR presents no or very little primary (non-induced) dormancy at harvest (MOMOH et al., 2002; GRUBER et al., 2004b), and develops secondary dormancy (thereafter referred to as dormancy) under specific conditions such as osmotic stress and darkness (PEKRUN et al., 1998). The disposition to dormancy is heritable, and the heritability was calculated to be 96–97% (SCHATZKI et al., 2013a; WEBER et al., 2013). In a group of 16 Canadian commercial OSR (Canola) varieties, the contribution of genotype to dormancy ranged from 44% to 82% (GULDEN et al., 2004a). Five QTLs have been recently found which explain 42% of the total dormancy in OSR (SCHATZKI et al., 2013b). Hence, selection of or breeding for low dormant varieties seems to be an effective strategy to reduce or to avoid soil seed banks of OSR and to prevent volunteers in the crop rotation. Particularly the increased – perceived or real – incidence of volunteer OSR in sown OSR could be controlled that way.

The mechanism of seed dormancy formation and heritability is complex, based on different physiological pathways and is not yet fully understood in many weeds and crops (BASKIN and BASKIN, 2005; FEI et al., 2007, 2009; FINKELSTEIN et al., 2008). The expression of seed dormancy is related to the genotype (FOLEY and FENNIMORE, 1998) and to maternal environmental conditions. Also in OSR, environmental conditions during growth and maturation of the seeds seem to affect the disposition to dormancy expression (GULDEN et al., 2004a; GRUBER et al., 2009; SCHATZKI et al., 2013a). For instance, the influence of the pre-harvest environment during seed maturation on seed dormancy accounted for 0.1–4.5% of total dormancy variation (GULDEN et al., 2004a).

Many OSR genotypes including genotypes with altered traits or ingredients have been analysed for dormancy in the past (GULDEN et al., 2003; GRUBER et al., 2004b; WEBER et al., 2010). However, nearly all results from dormancy were derived from open-pollinated varieties, and the dormancy of hybrids, particularly of imidazolinone-tolerant varieties (Clearfield®, CL), are not yet widely tested or tested at all, although CL OSR is being planted widely in Canada and the USA (BRIMNER et al., 2005). Furthermore, the differences between open-pollinated and hybrid OSR genotypes were seldom included in previous studies, and a possible segregation of dormancy in the F_2 generation of hybrid OSR was not yet analysed. Additionally, the effects of maternal environment on formation of potential seed dormancy and dormancy stability within population of a given genotype were not explicitly studied.

Therefore, the aim of the study was (i) to investigate for the first time a number of hybrid CL OSR genotypes, some of them launched in the European market, for their disposition to secondary dormancy in F_1 and F_2 generations, and (ii) to investigate impacts of the maternal environment on disposition to secondary dormancy.

Results are derived from two experimental approaches in the field: trial 1 with 15 genotypes grown at two locations; trial 2 with eight genotypes at 12 locations. Both varieties and breeding lines were used in this research, but in order to improve readability both of them were defined as "genotypes".

Materials and methods

Trial 1

The trial was performed in 2012/2013 as a randomized complete block design (four replicates) with 11 CL hybrid genotypes (imidazolinone-tolerant, Clearfield®) at the experimental station Ihinger Hof (IHO) of the University of Hohenheim and 15 CL hybrid genotypes at the location Hohenheim (Table 1).

Table 1. Oilseed rape genotypes (hybrids) used in two field trials, provided by three breeding companies. CL, Clearfield oilseed rape; non-CL, non-Clearfield oilseed rape. Trial 1: two locations; Trial 2: 12 locations, not presented
Rapsgenotypen (Hybridraps, von drei Züchtern), angebaut in zwei Feldversuchen. CL, Clearfieldraps; non-CL, kein Clearfieldraps.- Versuch 1 auf zwei Standorten, Versuch 2 auf 12 nicht genannten Standorten

Genotype		Trial 1		Trial 2
No.		Hohenheim	IHO	
1	CL	x	x	–
2	CL	x	x	–
3	CL	x	x	–
4	CL	x	x	–
5	CL	x	x	–
6	CL	x	x	–
7	CL	x	x	–
8	CL	x	–	–
9	CL	x	x	x
10	CL	x	x	x
11	CL	x	–	–
12	CL	x	–	–
13	CL	x	x	–
14	CL	x	–	–
15	CL	x	x	–
16	Non-CL	–	–	x
17	Non-CL	–	–	x
18	Non-CL	–	–	x
19	Non-CL	–	–	x
20	Non-CL	–	–	x
21	Non-CL	–	–	x

Both experimental locations were located in southwest Germany on a loamy soil and differed in precipitation and temperature, with 820 mm and 9.0°C for IHO and 710 mm and 9.8°C for Hohenheim; precipitation and temperature in the growing season of winter OSR in 2012/2013 at both locations is provided in Fig. 1. The CL OSR genotypes were sown at a density of 50 seeds m^{-2} with a plot size of 4 × 5 m for Hohenheim and 3×8 m for IHO on 27th and 29th August 2012, respectively. Weeds, pests and diseases were controlled according to the best management practice.

The main inflorescences of 10 randomly chosen individual plants of each plot at Hohenheim were isolated shortly before the beginning of the flowering stage using perforated plastic bags, ensuring aeration but excluding

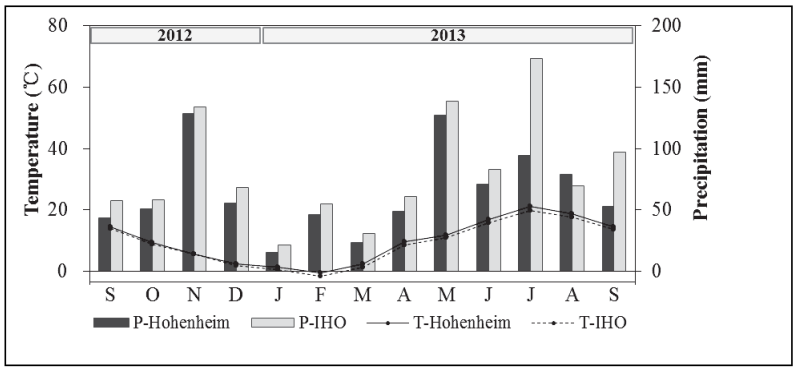

Fig. 1. Temperature (T, line) and precipitation (P, columns) in the growing season for winter oilseed rape in 2012/2013 at the locations of Hohenheim and Ihinger Hof (IHO).
Temperatur (T, Linie) und Niederschlag (P, Säulen) während der Anbauperiode von Winterraps 2012/2013 auf den Versuchsflächen Hohenheim und Ihinger Hof (IHO).

cross-pollination. The bags were lifted twice a week to provide enough space for the covered inflorescences to grow, and they were removed at early stage of pod formation. The beginning of flowering was noted on a label for each plant.

Hand harvest of the individual plants was done on 19th July 2013. The seeds from four out of the 10 isolated individual plants per plot with four replicates were harvested and kept separately (main stems from the individual plants; "IP") and the other six isolated plants were harvested and then mixed together by hand ("mixed-IP"). The remaining non-isolated plants in each plot were separately harvested by a plot combine harvester on 3rd August at IHO and 5thAugust 2013 at Hohenheim. After harvest, all the seed lots harvested by hand and by combine harvester were stored at room temperature, about 20°C, until the laboratory dormancy tests started.

The F_1 seeds of 15 CL genotypes were produced and harvested in 2011 by the seed companies. Genotypes 7–15 were treated with insecticides (thiamethoxam, active ingredient in Cruiser®) at delivery. All the seeds were stored under 10°C in an incubator until the dormancy test was conducted in February 2013. The F_2 seeds harvested in trial 1 were tested for dormancy and for viability during August and September 2013.

Hohenheim Standard Dormancy Test was used to test seed dormancy and viability (WEBER et al., 2010). The test comprises the induction of secondary dormancy on a polyethylene glycol solution (354.4 g in one l H_2O, –15 bar) in darkness (14 days), the identification of non-dormant seeds on water under darkness (seven days), and finally a viability test of potential dormant seeds under alternating light and temperature conditions (12 h darkness 3°C/12 h light 30°C, seven days). Finally, the test provides dormancy value as a percentage of the number of dormant seeds/viable seeds.

Trial 2

Two CL hybrid genotypes and six non-CL hybrid genotypes (Table 1) from one seed company were grown by the company itself at 12 locations in 2011/2012. Harvested seeds (F_2 generation) were analysed for dormancy by the Hohenheim Standard Dormancy Test in September 2012. The seeds were delivered to the authors for analysis and stored at room temperature until dormancy testing. Weather data in 2012 from the 12 stations nearest to the experiment locations was collected from http://www.dwd.de (DWD, Deutscher Wetterdienst, 2014).

Analysis

Arc-sin transformation was used to stabilize variance and adjust the data to a standard normal distribution using the following formula according to CHATTERJEE and HADI (2012),

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

where y, d and v are the transformed value, the absolute number of dormant seeds and the absolute number of viable seeds per replicate, respectively. All the adjusted data was back-transformed for presentation. The analysis of variance was performed by PROC MIXED of the statistical software SAS 9.3 (SAS Institute, Carey, NC, USA). In trial 1, all main effects for the three factors (genotype, location, and isolation of flowers) and their interactions were taken as fixed in the mixed model, while effects for replicate and the main plot were taken as random; in trial 2, effects of genotype, location and their interactions were taken as fixed, while only effects of lab replicates were taken as random because there were no field replicates at each location. Correlations coefficients were calculated in SAS by the procedure PROC CORR.

Results

Trial 1

The analysis of variance showed highly significant effects of genotype, location, plant isolation, and their interaction on seed dormancy (Table 2).

Dormancy varied between genotypes after artificial dormancy induction from 0.4 to 95.7% in the sown F_1 generation, from 4.1 to 86.9% in the F_2 offspring for mixed-IP (mixed individual, isolated plants) seeds at Hohenheim, and from 3.9 to 78.6% and from 9.3 to 76.6% for seeds from non-isolated plants at Hohenheim and IHO in the F_2 generations, respectively (Fig. 2). According to the variation of dormancy in the F_1 generation, genotypes could be classified into a low-dormant group (from genotype 1 to 9) with a mean value of 10.1% and high-dormant group (from genotype 10 to 15) with a mean value of 83.6%. F_1 and F_2 generations were strongly correlated concerning their seed dormancy potential with R = 0.96 and 0.91 for mixed-IP seeds and non-isolated seeds in the F_2 generation, respectively (Fig. 2).

The dormancy values of non-isolated seeds in the F_2 generation between the two locations Hohenheim and IHO were significantly correlated with R = 0.84 and 1.0 for low-dormant and high-dormant groups, respectively (Fig. 3). Seven out of eight genotypes (no data for genotype 8 at IHO) in the low-dormant group showed significant differences in seed dormancy between two locations (group a; Fig. 3), whereas no significant difference was detected in the high-dormant group (group b; Fig. 3).

The comparison between mixed-IP and non-isolated seeds at the same location brought significant differences for two out of nine low-dormant genotypes and for five out of six high-dormant genotypes (Fig. 4). The correlation between mixed-IP and non-isolated seeds at Hohenheim was significant with R = 0.97, although the dormancy value of non-isolated seeds was 6.9 percentage points lower than that of mixed-IP seeds.

The isolated individual (IP) plants varied in the mean dormancy of their offspring (Fig. 5). Plant-to-plant dormancy variation was 63.4–97.5%, 61.6–97.2%, 19.1–90.9%, 6.4–40.7%, 10.3–66.1% and 8.9–51.9% for genotype 15, 14, 10, 9, 8 and 3 with mean values of 88.8, 81.7, 62.6, 19.6, 35.9 and 31.3%, respectively.

Table 2. F-values for the effects of genotype, location, isolation, and their interaction on secondary dormancy of winter CL OSR genotypes in the F_2 generations (harvested seeds) in two trials; trial 1: 15 genotypes at two locations; trial 2: eight genotypes at 12 locations

F-Werte für die Effekte von Genotyp, Standort, Isolation des Blütenstandes (Selbstung) und deren Wechselwirkungen auf Samendormanz von CL Winterraps in der F_2 Generation (Erntegut) aus zwei Versuchen; Versuch 1: 15 Genotypen an zwei Standorten, Versuch 2: acht Genotypen an 12 Standorten

Field trial	Effect	DF	F-value	P
1	Genotype (G)	14	155.6	< 0.0001
	Location (L)	1	33.1	< 0.0001
	Isolation (I)	1	36.8	< 0.0001
	G×L	10	3.4	0.0003
	G×I	14	2.7	0.001
2	Genotype (G)	7	421.3	< 0.0001
	Location (L)	11	55.2	< 0.0001
	G×L	74	4.7	< 0.0001

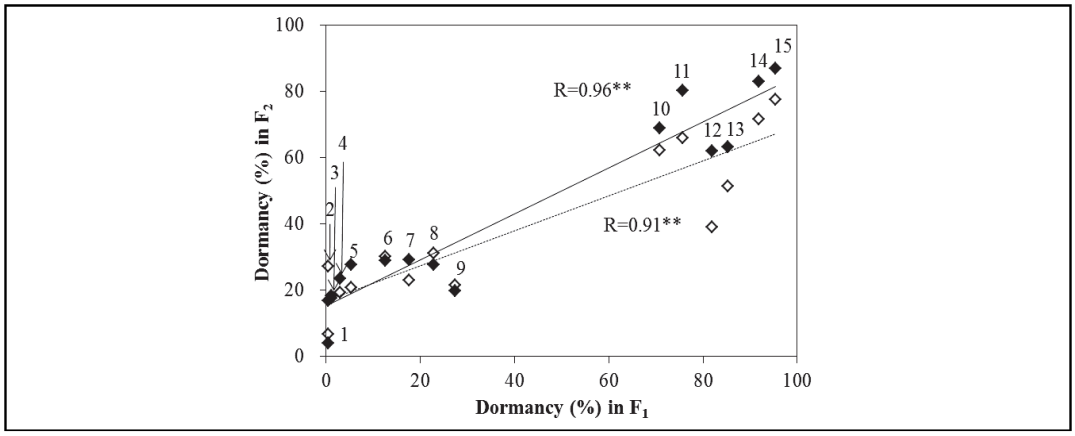

Fig. 2. Correlation of secondary dormancy (% dormant seeds/viable seeds after dormancy induction in the laboratory) of Clearfield oilseed rape between original hybrid seeds in F_1 and mixed isolated (♦ full line) and non-isolated seeds (◊ dashed line) in the F_2 generation. Isolated seeds of 15 genotypes in F_2 generation are derived from Hohenheim; dormancy values of non-isolated seeds of genotype 1–7, 9, 10, 13, 15 in F_2 generation are mean values across two locations, IHO and Hohenheim; genotype 8, 11, 12 and 14 in F_2 generation are from one location, Hohenheim. ** $P < 0.01$.

*Korrelation von sekundärer Dormanz (% dormante Samen/lebensfähiger Samen nach Dormanzinduktion im Labor) in Samen von Clearfieldrapssorten in Hybridsaatgut (F_1-Generation) und dem entsprechenden Erntegut (F_2-Generation; Mischung geselbsteter bzw. isolierter Einzelpflanzen je Sorte); ♦ durchgezogene Linie: Genotypen mit vergleichsweise niedriger Dormanzneigung; ◊ gepunktete Linie: Genotypen mit vergleichsweise hoher Dormanzneigung. Werte der F_2 für die frei abgeblühte Pflanzen der Genotypen 1–7, 9, 10, 13, 15 sind Mittelwerte aus den Standorten IHO und Hohenheim, Werte für die Genotypen 8, 11, 12, 14 nur vom Standort Hohenheim. ** $P < 0{,}01$.*

Field trial 2

Seed lots from two CL OSR and six non-CL OSR genotypes (Table 1) derived from 12 locations in Germany varied in dormancy depending on the location and on the genotype. The deviation in dormancy of a single genotype at a specific location from the mean of this genotype across all locations (location effects), was plotted against the deviation in dormancy of a single genotype at a specific location from the mean of this location across all genotypes (genotype effects; Fig. 6). The order of dormancy, from low to high, of these eight genotypes was genotype 17, 9, 16, 19, 10, 21, 18, and 20, and their mean dormancy levels across all locations were 42.1, 48.1, 67.7, 82.2, 84.3, 86.0, 87.9, and 90.0%, respectively (data not shown).

The closer a value is to the y-axis, the lesser the deviation of a genotype at this specific location is from the geno-

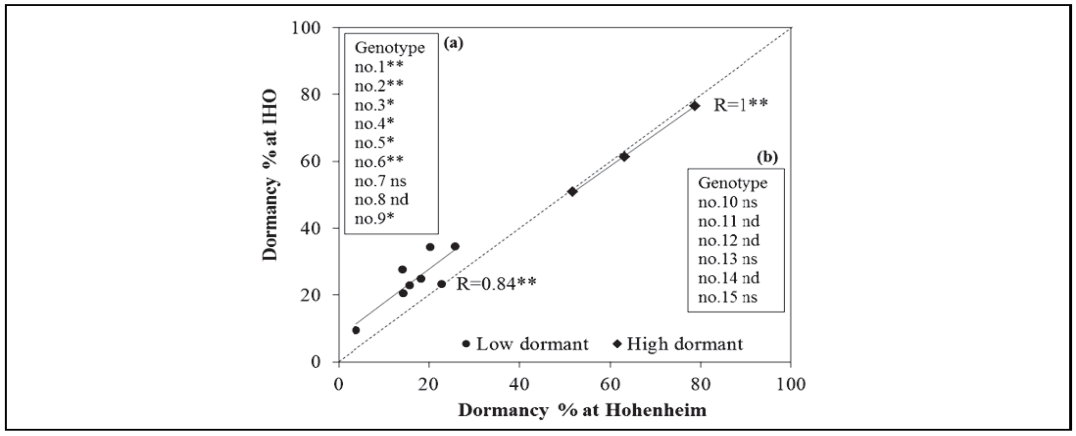

Fig. 3. Correlation of secondary dormancy (% dormant seed/viable seed after dormancy induction in the laboratory) in Clearfield oilseed rape seeds (• comparatively low dormant, ♦ comparatively high dormant) in the F_2 generation, harvested at the locations Hohenheim and IHO in 2013; the small frames (a, low dormant genotypes; b, high dormant genotypes) show the difference in dormancy between two locations of a given genotype. * $P < 0.05$; ** $P < 0.01$, ns not signifikant. Comparison within the same genotype at two locations; nd: no data available; dotted line represents the bisecting line of the graph.

*Korrelation von sekundärer Dormanz (% dormante Samen/lebensfähige Samen nach Dormanzinduktion im Labor) im Erntegut (F_2-Generation) von Clearfieldraps (• vergleichsweise niedrig dormante Genotypen, ♦ vergleichsweise hoch dormante Genotypen), geerntet auf den Standorten Hohenheim und Ihinger Hof im Jahr 2013. Die Kästchen (a, vergleichsweise niedrig dormante Genoytpen; b; vergleichsweise hoch dormante Genotypen) zeigen den Standortunterschied für die Dormanz des Erntegut jeweils eines Genotyps. * $P < 0{,}05$, ** $P < 0{,}01$, ns nicht signifikant; nd keine Daten verfügbar. Vergleich nur zwischen den Standorten innerhalb eines Genotyps.*

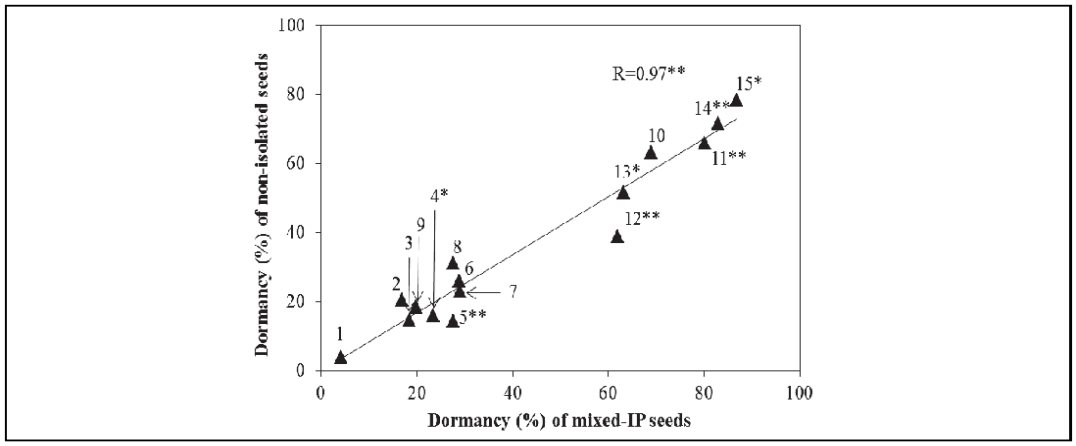

Fig. 4. Correlation of secondary dormancy (% dormant seeds/viable seeds after dormancy induction in the laboratory) in mixed-IP seeds and non-isolated seeds (both F_2 generation) of Clearfield oilseed rape, grown at the location Hohenheim. Mixed-IP: mixed seeds of isolated individual plants, non-isolated seeds: seeds from non-isolated plants. 1, 2 ... 15 are genotype numbers; * $P < 0.05$, ** $P < 0.01$, and ns not significant at $P < 0.05$; comparison within the same genotype between isolated seeds and mix-IP seeds.

*Korrelation von sekundärer Dormanz (% dormante Samen/lebensfähige Samen nach Dormanzinduktion im Labor) in Samen von Clearfieldraps aus dem gemischten Erntegut geselbsteter bzw. isolierter Einzelpflanzen („mixed-IP") und aus dem Erntegut offen abgeblühter Pflanzen („non-isolated seeds"; beides F_2-Generation) vom selben Standort (Hohenheim). * $P < 0{,}05$, ** $P < 0{,}01$, ns nicht signifikant bei $P < 0{,}05$.*

type mean across all locations. Regarding the locations, the closer a value is to the x-axis, the lesser the deviation of the location is from the location mean across genotypes. Locations with dormancy values close to the y-axis had led to less varietal variation. Genotypes with dormancy close to the y-axis responded comparatively little to location effects. Genotypes with lower dormancy (values below the x-axis) tended to respond more to effects

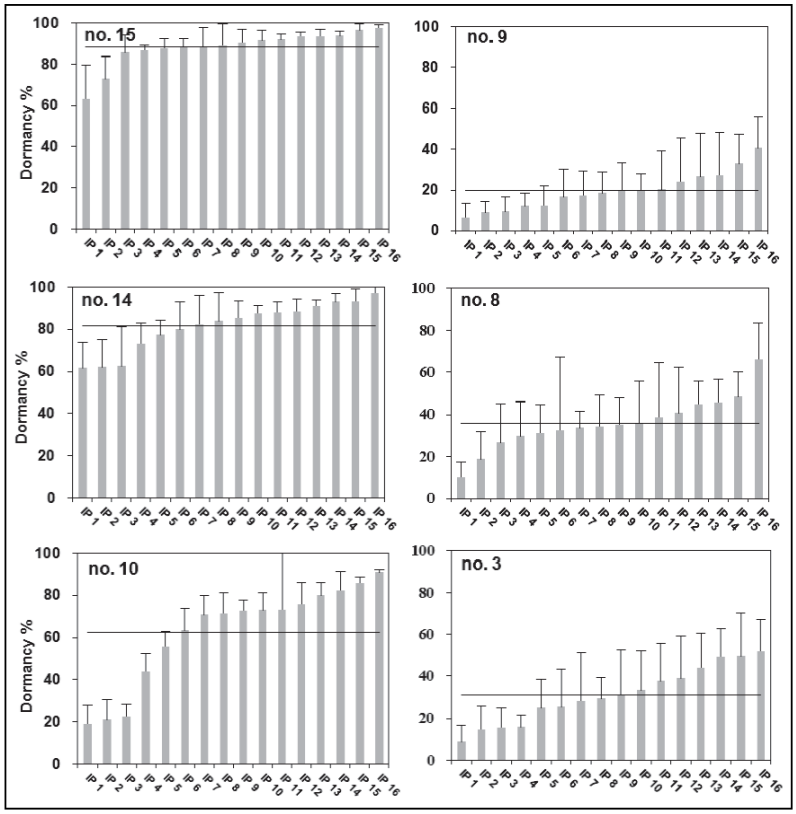

Fig. 5. Secondary seed dormancy (% dormant seed/viable seed after dormancy induction in the laboratory) in seeds harvested from 16 isolated individual plants (IP, four replicates of four individual plants) of six Clearfield oilseed rape genotypes (F_2 generation, harvest 2013, location Hohenheim). Horizontal lines stand for mean values of secondary dormancy (across individual IP-dormancy levels). Error bars = standard error of mean.
Sekundäre Dormanz (% dormante Samen/lebensfähige Samen) im Erntegut von 16 geselbsteten bzw. isolierten Einzelpflanzen (IP, vier Wiederholungen von vier einzelnen Pflanzen) von sechs verschiedenen Clearfieldraps-Genotypen (F_2-Generation, Ernte 2013, Standort Hohenheim). Horizontale Linien stehen für Mittelwerte der sekundären Dormanz aller geselbsteten Einzelpflanzen. Fehlerbalken = Standardfehler des Mittelwerts.

of the location than genotypes with higher dormancy. Location effects strongly correlated with genotype effects for genotype 9, 16 and 17, the dormancy levels of which were lower than the mean value of all genotypes at a specific location, with R = 0.95, 0.71 and 0.95, respectively (Fig. 6 A). Except genotypes 9, 16 and 17, other genotypes had higher dormancy levels but with lower correlation coefficient values between location effects and genotype effects, especially for highest dormant genotype 20.

The scatter plot distribution of Fig. 6 B indicates that locations 5, 8, 9 11 and 12 tended to reduce dormancy, and locations 1, 3, 6 and 10 tended to increase dormancy. The correlation coefficient (R) between location effects and genotype effects at the above locations was large and significant (positive or negative, R values in Fig. 6 B).

The amount of rainfall during ripening before harvest was positively correlated with the disposition to secondary dormancy, and the correlations became stronger with ripening period, while temperature showed negative correlations with dormancy but without significance (Table 3).

Discussion

Dormancy of hybrid CL oilseed rape

This study revealed a similar dormancy level and dormancy variation for 15 hybrid CL OSR genotypes in the F_1 and F_2 generations to that of non-CL OSR in previous studies (PEKRUN et al., 1997; GULDEN et al., 2003; GRUBER et al., 2004b; SCHATZKI et al., 2013a, b). In the research of GRUBER et al. (2004b), a dormancy variation of 3–76% among 32 non-CL OSR genotypes was detected, as well as a smaller range of 8–56% in a set of 28 black-seeded winter OSR genotypes measured by SCHATZKI et al. (2013a). The strong correlation between F_1 and F_2 generations in dormancy indicated that the disposition to secondary dormancy of hybrid CL OSR is heritable and robust (Fig. 2), but the correlation coefficient (R) was slightly lower than that of previous studies (SCHATZKI et al., 2013a; WEBER et al., 2013). First, seed storage time between genotypes in the F_1 generation was different, during which the disposition to seed dormancy would decrease (GRUBER et al., 2004b); second, the decrease of disposition to dormancy induction with time might be

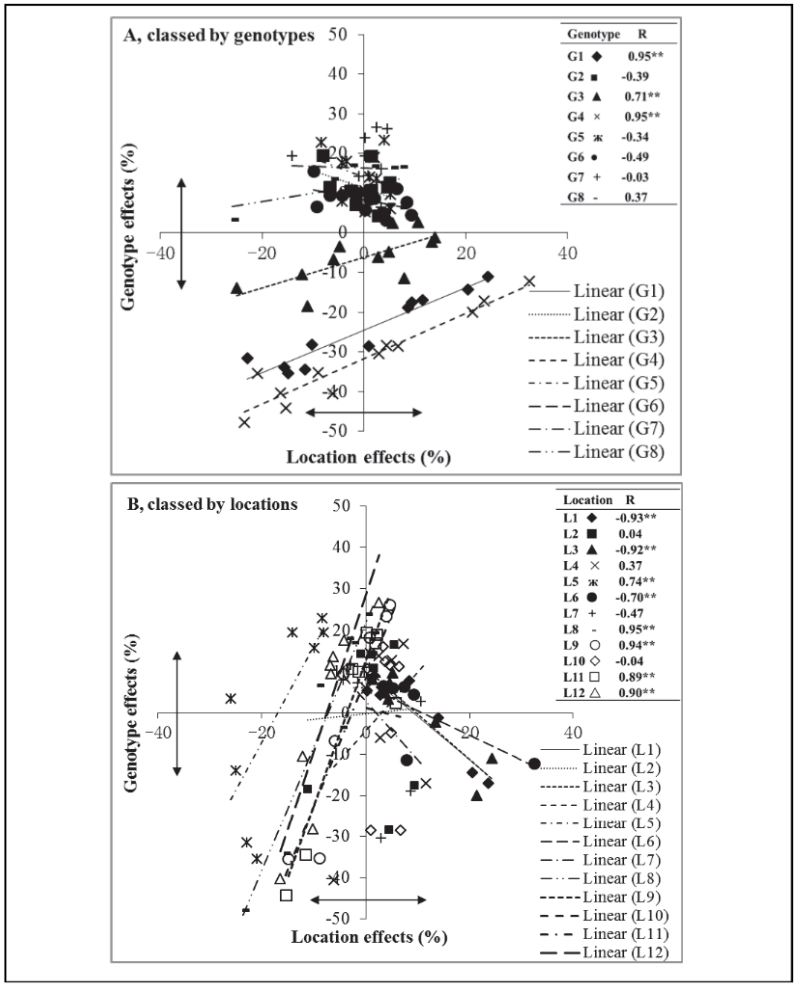

Fig. 6. Scatter plots of effects of genotype (A; n = 8) and location (B; n = 12) on the deviation of secondary seed dormancy (% dormant seed/viable seed after dormancy induction in the laboratory) of oilseed rape from the respective mean values across all genotypes and across all locations; Germany, 2012. Genotype 9 and 10 are Clearfield (CL) oilseed rape hybrids; genotypes 16 21 are non-CL hybrids. X-axis: deviation of the dormancy of a genotype at a specific location from the mean across all locations (indicating location effects); Y-axis: deviation of a genotype at a specific location from the mean across all genotypes on that location (indicating genotype effects).
Effekte von Genotyp (A; n = 8) und Standort (B; n = 12) auf die Abweichung der sekundären Dormanz (% dormante Samen/lebensfähige Samen nach Dormanzinduktion im Labor) in Rapssamen vom jeweiligen Mittelwert über alle Sorten bzw. über alle Standorte; Deutschland, 2012). Genotypen 9 und 10: Clearfieldraps-Hybriden, 16–21 nicht-Clearfieldraps. X-Achse zeigt Abweichung der Dormanz eines Genotyps an einem spezifischen Standorts von seinem Mittelwert über alle Standorte; Y-Achse zeigt Abweichung der Dormanz eines Genotyps an einem spezifischen Standort vom Mittelwert aller Genotypen an diesem Standort.

Table 3. Correlations between secondary dormancy of eight winter oilseed rape genotypes and average rainfall or air temperature (2 m) at different periods of ripening across 12 locations in Germany (weather data from DWD, 2014). * significant at $P < 0.05$, ** $P < 0.01$
*Korrelationen zwischen der Dormanzneigung von acht verschiedenen Winterraps-Genotypen und dem durchschnittlichen Niederschlag bzw. der Lufttemperatur (2 m) an unterschiedlichen Terminen während der Samenentwicklung an 12 Standorten in Deutschland (Wetterdaten nach DWD, 2014). * P < 0,05, ** P < 0,01*

	April	May	June	July	Aug.	June–July	June–Aug.	July–Aug.
Rainfall	0.06	−0.18	0.36	0.68*	0.60*	0.62*	0.72**	0.78**
Air temperature	−0.26	−0.41	−0.22	−0.34	−0.36	−0.28	−0.34	−0.40

different between genotypes of this study compared to previous studies; and third, the seed coating of genotypes 7–15 in the F_1 generation might have influenced dormancy induction and germination rates during the dormancy test. Overall, the strong genetic background to seed dormancy indicated that the dormancy level in the

F_2 generation which can cause volunteer problems can be determined through analyzing the dormancy potential of the F_1 generation (seeds to be used for sowing).

In spite of the strong correlation in dormancy between mixed-IP and non-isolated seeds (Fig. 4), the dormancy values of non-isolated seeds in most of studied hybrid CL genotypes were lower than that of mixed-IP seeds, especially for the high-dormant group. Outcrossing via pollen between genotypes might have caused the difference in dormancy between isolated and non-isolated seeds. Moreover, the potential effects of microclimate in the perforated plastic bags in the flowering period have to be taken into account. Hence, experiments using male sterile plants could quantify effects of outcrossing on dormancy variation.

Based on plant-to-plant dormancy variation within genotype, the dormancy segregation in the F_2 generation was obvious (Fig. 5), in accordance with findings of WEBER et al. (2013). The phenomenon of individual plants with different dormancy levels in their offspring is probably not only a segregation in the F_2 generation because WEBER et al. (2013) found this heterogeneity also in offspring from open pollinated varieties. The dormancy variation within the offspring seemed to depend on genotype: the mean range of dormancy variation across high dormant genotypes (no.14 and 15 except 10) was smaller than that of low dormant genotypes (no. 3, 8, and 9; 34.9% *vs.* 44.4%); the largest plant-to-plant variation (19.1–90.9%) was found in genotype No.10 with medium dormancy level (62.6%) in the F_2 generation. The limited number of genotypes does not yet allow stating a clear correlation between mean level of dormancy and segregation in the F_2. We hypothesize, however, that high dormant OSR genotypes are stable in their disposition to dormancy, and the range of dormancy in their offspring is smaller than in medium or low dormancy genotypes.

According to the similar results for the levels of dormancy in non-CL OSR in previous studies and in CL OSR in this study, soil seed bank and volunteer emergence of hybrid CL OSR are supposed to be similar to that of non-CL OSR. The very low-dormant genotypes identified in this study would probably result in low numbers or even no volunteers, if all other agricultural practices such as soil tillage are performed in an optimal way. Meanwhile, the large dormancy variation between and within genotypes could offer breeders strategies to select and breed low dormant CL OSR in coming years.

Impacts of maternal environment on dormancy disposition

Maternal environment is supposed to influence dormancy disposition of OSR during seed formation and maturation (GULDEN et al., 2004a; GRUBER et al., 2009; WEBER et al., 2013; POSTMA and ÅGREN, 2015). This was corroborated by the current study, e.g. the mean dormancy value across genotypes harvested at IHO was higher than that at Hohenheim in field trial 1. The most evident difference between two locations in trial 1was the high rainfall at IHO in July (Fig. 1) in the last period of ripening, which might be responsible for the different mean dormancy levels. The same phenomenon was observed in trial 2, e.g. locations with high rainfall during seed ripening had plants with higher dormancy level (significant correlation, Table 3).

The effects of locations, genotypes, and their interaction (Fig. 6) also indicate that seed dormancy of low dormant genotypes seems to be more prone to be influenced by maternal environment. Generally, there seem to be locations where maternal environmental conditions during seed maturation can result in higher disposition to dormancy of OSR. The amount of rainfall in the last period of ripening seems relevant for the disposition of OSR seeds to dormancy. Maybe wet conditions during ripening trigger physiological mechanisms which would prevent seeds from pre-harvest sprouting. Experiments with and analysis of phytohormones such as abscisic acid, which is clearly involved in the physiology of OSR dormancy (GULDEN et al., 2004b; FEI et al., 2009), would help to better understand the development of dormancy. Additionally, growing OSR plants under varied but controlled conditions of irrigation and temperature, for instance in climate chambers, would allow identifying environmental conditions which are crucial for the disposition to dormancy during seed ripening of OSR.

Conclusion

Hybrid CL oilseed rape genotypes show dormancy levels similar to those of non-CL genotypes, based on the comparison to previous results. Selection of or breeding for low-dormant OSR seems feasible based on dormancy variability between and within genotypes, and based on the fact that dormancy values of the F_2 generation correspond to that of the hybrid F_1 generation, at least in the mean of the offspring. There exist locations that obviously allow higher segregation of the genotypes, so that low dormant genotypes can be more easily detected. It is still not known which maternal environmental factors actually influence the disposition to dormancy and what is the physiological background; precipitation seems to be one factor.

Acknowledgements

The authors want to thank Andrea RÜCKLE for excellent technical support, to thank the staff of the experimental station Ihinger Hof for the excellent field management, and also to thank the support of China Scholarship Council (CSC).

References

BASKIN, C.C., J.M. BASKIN, 2005: Underdeveloped embryos in dwarf seeds and implications for assignment to dormancy class. Seed Science Research **15**, 357-360.

BRIMNER, T.A., G.J. GALLIVAN, G.R. STEPHENSON, 2005: Influence of herbicide- resistant canola on the environmental impact of weed management. Pest Management Science **61**, 47-52.

CHATTERJEE, S., A.S. HADI, 2012: Regression analysis by example, 5th ed., Hoboken, NJ, John Wiley & Sons, pp. 163-185.

D'HERTEFELDT, T., R.B. JØRGENSEN, L.B. PETTERSSON, 2008: Long-term persistence of GM oilseed rape in the seedbank. Biology Letters **4**, 314-317.

DWD, 2014: Wetter und Klima - Deutscher Wetterdienst, DWD. http://www.dwd.de/(access10/2014).

FEI, H., Y. FERHATOGLU, E. TSANG, D. HUANG, A.J. CUTLER, 2009: Metabolic and hormonal processes associated with the induction of secondary dormancy in *Brassica napus* seeds. Botany **87**, 585-596.

FEI, H., E. TSANG, A.J. CUTLER, 2007: Gene expression during seed maturation in *Brassica napus* in relation to the induction of secondary dormancy. Genomics **89**, 419-428.

FINKELSTEIN, R., W. REEVES, T. ARIIZUMI, C. STEBER, 2008: Molecular Aspects of Seed Dormancy*. Plant Biology **59**, 387.

FOLEY, M.E., S.A. FENNIMORE, 1998: Genetic basis for seed dormancy. Seed Science Research **8**, 173-182.

GRUBER, S., C. PEKRUN, W. CLAUPEIN, 2004a: Population dynamics of volunteer oilseed rape (*Brassica napus* L.) affected by tillage. European Journal of Agronomy **20**, 351-361.

GRUBER, S., C. PEKRUN, W. CLAUPEIN, 2004b: Seed persistence of oilseed rape (*Brassica napus*): variation in transgenic and conventionally bred cultivars. The Journal of Agricultural Science **142**, 29-40.

GRUBER, S., K. EMRICH, W. CLAUPEIN, 2009: Classification of canola (*Brassica napus*) winter cultivars by secondary dormancy. Canadian Journal of Plant Science **89**, 613-619.

GÜLDEN, R.H., S.J. SHIRTLIFFE, A.G. THOMAS, 2003: Secondary seed dormancy prolongs persistence of volunteer canola in western Canada. Weed Science **51**, 904-913.

GÜLDEN, R.H., A.G. THOMAS, S.J. SHIRTLIFFE, 2004: Relative contribution of genotype, seed size and environment to secondary seed dormancy potential in Canadian spring oilseed rape (*Brassica napus*). Weed Research **44**, 97-106.

LUTMAN, P.J.W., K. BERRY, R.W. PAYNE, E. SIMPSON, J.B. SWEET, G.T. CHAMPION, M.J. MAY, P. WIGHTMAN, K. WALKER, M. LAINSBURY, 2005: Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (*Brassica napus*). Proceedings of the Royal Society B: Biological Sciences **272**, 1909-1915.

LUTMAN, P.J.W., S.E. FREEMAN, C. PEKRUN, 2003: The long-term persistence of seeds of oilseed rape (*Brassica napus*) in arable fields. The Journal of Agricultural Science **141**, 231-240.

MESSÉAN, A., C. SAUSSE, J. GASQUEZ, H. DARMENCY, 2007: Occurrence of genetically modified oilseed rape seeds in the harvest of subsequent conventional oilseed rape over time. European Journal of Agronomy **27**, 115-122.

MOMOH, E.J.J., W.J. ZHOU, B. KRISTIANSSON, 2002: Variation in the development of secondary dormancy in oilseed rape genotypes under conditions of stress. Weed Research **42**, 446-455.

PEKRUN, C., P. LUTMAN, K. BAEUMER, 1997: Induction of secondary dormancy in rape seeds (*Brassica napus* L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. European Journal of Agronomy **6**, 245-255.

PEKRUN, C., J.D.J. HEWITT, P.J.W. LUTMAN, 1998: Cutltural control of volunteer oilseed rape (*Brassica napus*). The Journal of Agricultural Science **130**, 155-163.

POSTMA, F.M., J. ÅGREN, 2015: Maternal environment affects the genetic basis of seed dormancy in Arabidopsis thaliana. Molecular Ecology **24**, 785-797.

SCHATZKI, J., B. SCHOO, W. ECKE, C. HERRFURTH, I. FEUSSNER, H.C. BECKER, C. MOLLERS, 2013a: Mapping of QTL for seed dormancy in a winter oilseed rape doubled haploid population. Theoretical and Applied Genetics **126**, 2405-2415.

SCHATZKI, J., M. ALLAM, C. KLÖPPEL, M. NAGEL, A. BÖRNER, C. MÖLLERS, A.M. CHEVRE, 2013b: Genetic variation for secondary seed dormancy and seed longevity in a set of black-seeded European winter oilseed rape cultivars. Plant Breeding **132**, 174-179.

WEBER, E.A., K. FRICK, S. GRUBER, W. CLAUPEIN, 2010: Research and development towards a laboratory method for testing the genotypic predisposition of oilseed rape (*Brassica napus* L.) to secondary dormancy. Seed Science and Technology **38**, 298-310.

WEBER, E.A., S. GRUBER, W. CLAUPEIN, 2014: Emergence and performance of volunteer oilseed rape (*Brassica napus*) in different crops. European Journal of Agronomy **60**, 33-40.

WEBER, E.A., S. GRUBER, F. STOCKMANN, W. CLAUPEIN, 2013: Can low-dormancy oilseed rape (*Brassica napus*) genotypes be used to minimize volunteer problems? Field Crops Research **147**, 32-39.

5 Chapter III

Field history of imidazolinone-tolerant oilseed rape (*Brassica napus*) volunteers in following crops under six long-term tillage systems.

Publication III

Huang, S., Gruber, S., Claupein, W. (2016):
Field history of imidazolinone-tolerant oilseed rape (*Brassica napus*) volunteers in following crops under six long-term tillage systems.
Field Crops Research

After harvest of oilseed rape, soil seed bank and occurrence of volunteers are strongly dependent on mode of tillage, operation time and variety. Between volunteers of imidazolinone-tolerant oilseed rape, gene segregation in herbicide tolerance is supposed to occur. Against this background, tillage strategy along with choice of variety are supposed to reduce soil seed bank size and the number of volunteers. The quantification of gene segregation could be measured in the volunteers. This chapter confirmed some previous findings that varieties with high seed dormancy or early soil disturbance after harvest of oilseed rape can result in larger soil seed banks, and non-inversion tillage can result in more volunteers. Interestingly, release from dormancy might be different between varieties resulting in the difference in volunteer emergence. Gene segregation will reduce the tolerance of volunteers to acetolactate synthase inhibiting herbicides.

Chapter III: Field history of imidazolinone-tolerant oilseed rape (*Brassica napus*) volunteers in following crops under six long-term tillage systems.

Field Crops Research 185 (2016) 51–58

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Field history of imidazolinone-tolerant oilseed rape (*Brassica napus*) volunteers in following crops under six long-term tillage systems

Shoubing Huang*, Sabine Gruber, Wilhelm Claupein

University of Hohenheim, Institute of Crop Science, Fruwirthstr. 23, 70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 23 July 2015
Received in revised form 27 October 2015
Accepted 27 October 2015
Available online 9 November 2015

Keywords:
Canola
Dormancy
Clearfield
Soil inversion
Soil seed bank
Genetic analysis

ABSTRACT

Volunteer oilseed rape (OSR, *Brassica napus* L.) exhibits weedy behavior in crops, and can contribute to gene flow or unwanted seed admixture, particularly if its variety is tolerant to specific herbicides and if the proportion of OSR in a crop rotation is high. The aim of this study was to monitor the fate of seeds of imidazolinone-tolerant oilseed rape (Clearfield®; CL OSR) lost at harvest over the two years following its intentional sowing. A 5-yr experiment (2011–2015) with non-CL OSR and CL OSR in the same rotation was conducted on an existing long-term tillage experiment in south-west Germany to investigate OSR volunteer dynamics. The experiment included different modes of primary tillage (inversion tillage, non-inversion tillage, no-till, with or without additional stubble tillage prior to primary tillage). The crop sequence was non-CL OSR, winter wheat (*Triticum aestivum* L.), CL OSR (a medium and a high dormancy variety), winter wheat, and maize (*Zea mays* L.). High dormancy CL OSR resulted in a larger soil seed bank (147 vs. 58 seeds m^{-2}), but in fewer volunteers (0.9 vs. 1.9 volunteers m^{-2}) than the medium dormancy variety in the first year after CL OSR. Dormancy release likely resulted in different volunteer emergence rates of the two varieties. Immediate stubble tillage after CL OSR increased seed bank and volunteers by 0.93 and 12.7 times, respectively. Inversion tillage resulted in 30 times fewer volunteers in the first year after CL OSR, but in an equal volunteer number in the second year compared to non-inversion tillage. Slight segregation of imidazolinone-tolerant genes occurred in the offspring of CL OSR, likely leading to different CL herbicide-tolerance levels in volunteers though the number of these individuals was small.

Sound strategies to control OSR volunteers should include (1) use of low dormancy varieties with low potential to establish a seed bank, (2) varieties with fast dormancy release to trigger more dormant seeds to germinate in a very short period, and (3) a period of time between harvest of OSR and first tillage operation to reduce the possibility of seeds entering soil seed bank.

1. Introduction

High seed loss before and during harvest combined with the potential of seeds to establish a soil seed bank can make oilseed rape (*Brassica napus*; OSR) volunteers weeds in following crops. In particular, volunteers of herbicide tolerant OSR such as imidazolinone-tolerant OSR (Clearfield®, CL OSR) cannot be controlled by herbicides with the same mode of action. Currently, the introduction of CL OSR into Europe poses new challenges for chemical control of CL volunteers because of their tolerance to common acetolactate synthase (ALS) inhibiting herbicides. For chemical control of these volunteers, other herbicides would be necessary or non-chemical methods as alternatives. Farmers would probably adopt CL OSR mainly because of an expected large spectra efficacy of associated herbicides for general weed control (e.g. post-emergence weed control). The planting of CL OSR, however, could also be a strategy to remove volunteers from non-CL varieties from the field and to obtain a pure seed lot at harvest without any admixture from old varieties, such as e.g. those with unwanted patterns of fatty acids.

The harvest loss of OSR can reach up to 10 000 seeds m^{-2} (Lutman et al., 2005) depending on ripening (Zhu et al., 2012) and harvesting conditions (Price et al., 1996; Peltonen-Sainio et al., 2014). Approximately 1–29% of these shed seeds can fall into secondary dormancy (hereafter referred to as dormancy) and enter the soil seed bank, if specific post-harvest environmental conditions occur such as osmotic stress, darkness, or oxygen deficiency (Pekrun et al., 1997; Momoh et al., 2002), depending on soil tillage and genetic disposition of the respective varieties to dormancy

* Corresponding author. Fax: +49 71145923773.
E-mail addresses: shuang@uni-hohenheim.de (S. Huang), Sabine.Gruber@uni-hohenheim.de (S. Gruber), Wilhelm.Claupeim@uni-hohenheim.de (W. Claupein).

http://dx.doi.org/10.1016/j.fcr.2015.10.019
0378-4290/

(Gruber et al., 2005, 2010; Lutman et al., 2005). Non-inversion and no-till treatments often resulted in higher emergence of OSR volunteers in the first following year than inversion tillage due to their effects on seed distribution in the soil (Gulden et al., 2003; Gruber et al., 2005). Moreover, tillage can influence micro-environmental and edaphic conditions in the soil, such as water content, light penetration, temperature, and oxygen concentration (Clements et al., 1996) which can determine seed survival, dormancy release, and germination (Pekrun et al., 1997; López-Granados and Lutman, 1998; Gulden et al., 2004a).

Dormancy characteristics were considered to be one crucial factor in determining soil seed bank and OSR volunteers in the following crops. Gulden et al. (2003, 2004a) reported that in Canada high dormancy spring OSR varieties resulted in about 6- to 12-fold greater seed persistence than low dormancy varieties, which is consistent with results of winter OSR from Gruber et al. (2010) in Germany. Thus, dormant seeds buried in the soil seed bank can persist over several years and then germinate, causing a long-term volunteer problem in the following crops (Lutman et al., 2003; Gruber et al., 2004b). Volunteers can be unwanted not only during the production of OSR seeds for consumption but rather also for seed multiplication. Official regulations of the European Community dealing with the marketing certified seeds require that "the field shall be sufficiently free from such plants which are volunteers from previous cropping" (Council Directive, 2002), to maintain seed purity for seed multiplication. However, even after a rotational break of eight years, volunteers can occur in the next sown OSR (Messéan et al., 2007). Seed impurities due to admixture with OSR varieties from preceding crops amounted for up to 19% in that study but the proportion of impurities due to volunteers was not closely linked with the duration of the break. Therefore, additional reasons must exist for some varieties to persist in high amounts and for a long time.

The effects of varieties with different dormancy levels on volunteer number has been seldom referred to, although it has been reported that high dormancy varieties can increase volunteer numbers in the following crops over the long-term, irrespective of tillage system, crop competition, and climate factors (Gulden et al., 2003; Gruber et al., 2004c; Weber et al., 2014). In addition, the OSR varieties used in the above studies were mostly open pollinated varieties and non-CL varieties. Little has been published about CL OSR hybrids in terms of seed dormancy, seed bank, and volunteers, though CL OSR has been planted widely in Canada and the USA (Brimner et al., 2005). There is also little information about the fate of seeds from high and low dormancy OSR varieties under different tillage treatments at the same location, information which would help to identify whether variety or tillage is more crucial for controlling volunteers. Furthermore, there is little information about how many volunteers derive from CL oilseed rape, or on how many have emerged from the soil seed bank of previously grown non-CL OSR.

The objectives of this study were (i) to track the evolution of CL and non-CL OSR volunteers in a crop sequence with 50% OSR in different tillage systems on; (ii) to examine varietal differences in the fate of a medium and a high dormancy CL OSR variety in the seed bank and in volunteer performance under six tillage treatments.

2. Materials and methods

2.1. Site characteristics and experimental design

The trial was conducted at an existing long-term experimental site begun in 1999 at the experimental station "Ihinger Hof" (IHO) of the University of Hohenheim, SW Germany (48°45′N, 8°56′E), on a loamy soil, with annual precipitation and annual temperature of 690 mm and 7.9 °C, respectively.

The monitored crop sequence from 2010 to 2015 in the field trial was winter non-CL OSR (*B. napus*)/winter wheat (*Triticum aestivum*)/winter CL OSR (*B. napus*; two anonymous varieties)/winter wheat (*T. aestivum*)/maize (*Zea mays*; Table 1). The two CL OSR varieties had a medium (variety 1) or high level of secondary seed dormancy (variety 2), and both were hybrids with genes for imidazolinone-tolerance, PM_1 and PM_2 genes being homozygous for both varieties (ho/ho).

A one factorial tillage trial was set up in 2010–2011 on an existing field trial area (long- term tillage, Gruber et al., 2010). The trial was arranged in a randomized complete block design with a plot size of 18 × 50 m (4 replicates). In autumn 2011, the plots were split into two halves and two CL OSR varieties were sown on each half, so that the experiment became a split-plot design with tillage as main factor (six levels) and variety (two levels) as second factor with a sub-plot size of 9 × 50 m. Six long-term tillage systems were involved in the recent research with different modes of primary tillage (or no-till) combined or not combined with stubble tillage (Table 2).

2.1.1. Herbicide application

Herbicides were applied according to best management practice in the years 2010–2013 (non-CL oilseed rape, winter wheat, CL-oilseed rape); the herbicide Vantiga plus Dash was used for the CL varieties (Table 3). During the growing period of winter wheat (2013/2014) no herbicide was sprayed in order to simulate the worst case of CL OSR volunteers and to observe the effects solely of tillage and variety on the number of volunteers.

2.2. Sampling and data collection

2.2.1. Determination of OSR volunteers in 2011–2015

Non-CL OSR volunteers were counted using a frame of 0.1 m^2 10 times per plot in the period between harvest of OSR Avatar in 2011 and sowing of winter wheat on 21/09/2011 (Counting 1, Table 4). At that time, stubble tillage had been done in SP, SC, and SR, but primary tillage had not been performed on all the treatments. OSR volunteers were counted in winter wheat on 07/03/2012 (Counting 2), and after harvesting of winter wheat on 22/08/2012 between stubble tillage and primary tillage (Counting 3), both using the same method as in Counting 1. In the second following crop, CL OSR, supposed non-CL volunteers were counted again on 25/04/2013 (Counting 4) between rows (row spacing 0.17 m) within a 2 m length with four replicates per plot. In winter wheat following CL OSR, all flowering volunteers per plot were marked with different color-coded labels (depending on the onset of flowering) on April 14, April 25, May 12, and May 21 2014 to monitor the course of flowering, and were counted (Counting 5). The emerged volunteer per plot was counted in maize on 26/05/ 2015 (Counting 6) before any weed control (i.e. before herbicide application).

2.2.2. Seed loss of CL OSR in autumn 2013

Only seed loss from CL OSR was determined. Plots were harvested by a plot combine harvester on 01/08/2013, then seed loss in the sampling area of 0.2 × 1.0 m was collected on August 01 and 02 by a vacuum cleaner (500 w) with 4 replicates per plot. To avoid germination, the samples were dried immediately after collection at 40 °C for 48 h. The seeds were cleaned and picked manually from the samples.

2.2.3. Soil seed bank of OSR in spring 2014

Soil sampling to determine the soil seed bank took place on 05/03/2014, in three depths 0–10, 10–20, and 20–30 cm. Forty soil

Chapter III: Field history of imidazolinone-tolerant oilseed rape (*Brassica napus*) volunteers in following crops under six long-term tillage systems.

Table 1
Crop sequence of a long-term tillage field trial including two years oilseed rape, varieties and sowing density.

Period	06/09/2010–27/07/2011	12/10/2011–01/08/2012	29/08/2012–02/08/2013	28/11/2013–04/08/2014	24/04/2015–17/09/2015
Crop sequence	Winter OSR	Winter wheat	CL OSR	Winter wheat	Maize
Variety	Avatar	Meister	Anonym.	Patras	Frederico
Seeds m^{-2}	40	300	40	380	9.5

Table 2
Tillage treatments and operation time (dd/mm) for a long-term field trial in 2011–2014. Stubble tillage was performed immediately after harvest[a]. SP, P: conventional tillage; SC, C, SR: conservation tillage; NT: no tillage.

Treatment	Stubble tillage (S)	Implement of primary tillage	2011		2012		2013		2014	
			S	P	S	P	S	P	S	P
Inversion tillage										
SP (20 cm)	Yes	Mouldboard plough	01/08,12/09	11/10	03/08	28/08	10/08	25/11	05/09	20/10
P (20 cm)	No	Mouldboard plough		11/10		28/08		25/11		20/10
Non-inversion tillage										
SC (18 cm)	Yes	Chisel plough	01/08,12/09	11/10	03/08	27/08	10/08	26/11	05/09	20/10
C (18 cm)	No	Chisel plough		11/10		27/08		26/11		20/10
SR (10 cm)	Yes	Rototiller	01/08	11/10	03/08	29/08	10/08	27/11	05/09	20/10
NT	No	No tillage								

[a] Stubble tillage in SP and SC was conducted twice by a chisel plough in 2011, in a depth of 6–8 cm (01/08) and 11 cm (12/09/2011), respectively; in 2012–2014 all stubble tillage was performed in a depth of 6–8 cm for SP and SC by a chisel plough, and 4–6 cm for SR by a rototiller.

Table 3
Herbicides and application time (dd/mm) for the long-term trial in 2011–2014; no herbicides were applied in the growing season of winter wheat from November 2013 to August 2014; tillage treatments see Table 2.

Tillage	Application time and herbicides			
	2011	2012	2013	2014
Inversion tillage				
SP	23/09Clinic[a]	10/04 Mix[b] 14/09 Vantiga + Dash [c])	24/10 Glyph.[d]	14/10 Glyph.[e]
P	23/09"	10/04, 14/09"	24/10"	14/10"
Non-inversion tillage				
SC	23/09"	10/04, 14/09"	24/10"	14/10"
C	23/09"	10/04, 14/09"	24/10"	14/10"
SR	23/09"	10/04, 14/09"	24/10"	14/10"
NT	23/09"	10/04[b], 23/08[d], 14/09[c]	24/10"	14/10"

[a] Clinic 3 l ha^{-1}.
[b] 350 g ha^{-1} Atlantis, 0.7 l ha^{-1} FHS, 80 g ha^{-1} Alliance, 75 ml ha^{-1} Primus, and 0.5 l ha^{-1} Cycocel.
[c] Vantiga 2 l ha^{-1}, Dash 1 l ha^{-1}.
[d] Glyphosate 4 l ha^{-1}.
[e] Glyphosate 4 l ha^{-1}.

Table 4
Times (dd/mm/yy) and methods to determine oilseed rape (OSR) volunteers (non-CL and CL) in a crop sequence over five years with different tillage treatments 2011–2015. WW: winter wheat, CL Clearfield; crop sequence described in Table 1.

	Time	Period of crop growth	Monitoring methods
	Non-CL volunteers in 2011–2013		
Counting 1	21/09/2011	Between stubble and primary tillage; before sowing of WW	Frame[a]
Counting 2	07/03/2012	In WW	Frame
Counting 3	22/08/2012	Between stubble and primary tillage; before sowing of CL OSR	Frame
Counting 4	25/04/2013	In CL OSR	Rows[b]
	CL (and non-CL) volunteers in 2014–2015		
Counting 5	14/04–21/05/2014	Flowering volunteers in WW	Whole plot
Counting 6	26/05/2015	In maize	Whole plot

[a] 0.1(frame) × 10 m^2.
[b] 0.17 (row spacing) × 2 (length) × 4 m^2.

samples were taken randomly in each plot by an auger with diameter of 11 × 15 mm, and merged to obtain one plot sample per each of the three sampling depths, and then immediately deep-frozen for storage until analysis. Two sieves with mesh widths of 4 mm and 1 mm were used to wash out and separate OSR seeds from defrosted soil samples in water. The number of intact seeds m^{-2} with yellow embryos was regarded as the OSR soil seed bank.

2.2.4. Genetic analysis in 2014

To identify the non-CL and CL volunteers in the following crop winter wheat in 2014, up to 20 flowering volunteer plants were randomly selected in each plot, and 2–4 discs of 6 mm diameter were cut from the youngest leaflets of the volunteers with Harris UNICORETM in April 2014. If the volunteer number in the plot was less than 20 plants, all volunteers were used. Afterwards, the leaf discs

of each plant were dried and stored in tubes of a 96-well-plate, and sent to an external laboratory (ScanBi Diagnostics, Arnalb, Sweden) for DNA analysis. DNA extraction from leaf discs and genetic analysis were performed using PCR and pyrosequencing by an external laboratory, similar to Laufer et al. (2014). Imidazolinone-tolerant gene BnAHASL1C (target of PM_1 resistance) and BnAHASL1A (target of PM_2 resistance) were the target lines for genetic analysis.

2.2.5. Seed dormancy of non-CL and CL varieties and volunteers

OSR variety Avatar was routinely tested for dormancy level by the standard dormancy test (Weber et al., 2010) and dormancy accounted for 64% (independent variety test, from harvest of 2014 on other fields; data not shown).

Seed dormancy of the two CL OSR varieties (F_1 generation, used for sowing) were tested in March 2013, more than eight months after sowing. Shortly before the harvest, fresh seeds (F_2 generation) in the main inflorescences of 10 individual plants of two varieties in each plot were harvested randomly by hand in August 2013, and then were mixed and tested for dormancy in September 2013. The seeds harvested by combine harvester were tested in November 2013, after a storage period of approximately four months at 20 °C.

All the volunteers in winter wheat were harvested by hand on 31/07/2014 shortly before harvest of winter wheat to determine whether they were derived from the CL OSR or from prior growing of variety Avatar, or from outcrossing. The harvested volunteer plants were stored at 20 °C. Seven months later, volunteer seeds were threshed in February 2015. Seed dormancy values were determined exemplary only from volunteers in tillage treatment of SC, independently from their time of flowering.

Seed dormancy in seeds of all generations was tested with the Hohenheim standard dormancy test (Weber et al., 2010). In this method, 100 seeds were soaked in darkness at 20 °C in a 6 ml PEG 6000 solution (−15 bar) in each petri dish on double filter paper for 14 days for dormancy induction (four replicates), followed by 14 days in deionized water in darkness for seed germination, and then by 7 days in alternating light and temperature (12 h light 30 °C/12 h dark 3 °C) for dormancy breaking. Dormancy value was expressed as the percentage of induced dormant seeds from total number of viable seeds per accession.

2.2.6. Statistical analysis

The analysis of variance of seed loss, volunteers, proportion of volunteers to seed loss (vo./seed loss) and seed dormancy of CL volunteers was performed by PROC MIXED of the statistical software SAS 9.3 (SAS Institute, Carey, NC, USA). PROC GLIMMIX was used to analyze the variance of seed bank and proportion of seedbank to seed loss (SSB/seed loss). In the above model procedures, the effects of variety, tillage, and their interactions were taken as fixed with effects for replicate as random. Significances between varieties or tillage treatments were determined by a *t*-test ($\alpha < 0.05$) with Satterthwaite's approximation.

3. Results

3.1. Non-CL OSR volunteers in the following crops in 2011–2013

Before sowing winter wheat in 2011, a range of 10–473 plants m^{-2} of non-CL volunteers, most of which probably came from harvest in 2011, was recorded in the time between stubble tillage and primary tillage (Counting 1, Table 5).

A certain number of OSR seeds from the 2011 harvest seemed to have survived in the soil seed bank and some of them emerged in the growing season of winter wheat from autumn 2011 to spring 2012 (with usual herbicide application). Only treatments C and NT had volunteers, in numbers of 0.50 and 0.25 plants m^{-2}, respectively, before herbicide application in spring 2012 (Counting 2,

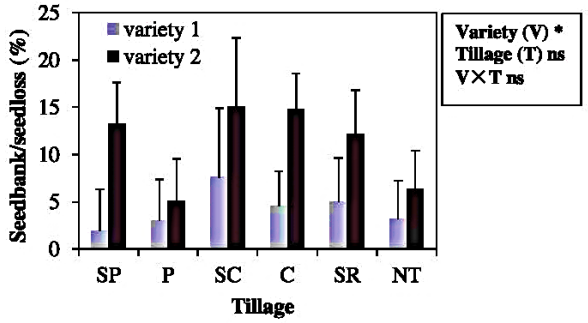

Fig. 1. Proportion of the soil seed bank (seeds m^{-2}) of Clearfield oilseed rape to seed loss m^{-2} during harvesting in the first spring (2014) after harvest (2013), as an effect of variety (variety 1, medium dormancy; variety 2, high dormancy) and tillage (see Table 2); * Significant at $P < 0.05$, ns: not significant; error bars: standard error of mean.

Table 5). After harvest of WW and before sowing of the following two CL OSR varieties, a range of 0.0–3.5 plants m^{-2} of non-CL volunteers between stubble and primary tillage were recorded on 22/08/2012 (Counting 3, Table 5). In the sown CL OSR (Counting 4, Table 5), there were at least 0.6 supposed non-CL volunteers m^{-2} (plants between rows) found in spring 2013 shortly before CL OSR started flowering (in treatment P), and a maximum of 2.2 volunteers m^{-2} (in treatment SR); Vantiga was applied in autumn before. Treatments with stubble tillage (SP, SC, and SR) had more volunteers than those without stubble tillage in Counting 3 and 4, Table 5.

3.2. Harvest seed loss and seed bank of CL OSR in 2013–2014

The tillage systems significantly affected seed loss of CL OSR during harvest, and the variety had a significant effect on the size of the soil seed bank and on the proportion of seed bank to seed loss of CL OSR in WW (Table 6).

Seed loss of CL OSR ranged from approximately 760 to 2490 seeds m^{-2} with a mean of 1530 across tillage treatments and varieties (Table 7). Variety 1 had nearly the same mean seed loss as variety 2 had (1540 seeds m^{-2}) across six tillage treatments, but resulted in a soil seed bank more than twice as large as the soil seed bank of variety 2 (147 vs. 58 seeds m^{-2}, Table 7). On average across both varieties, inversion tillage (mean of SP and P) resulted in a soil seed bank approximately as big as from non-inversion tillage (100 vs. 103 seeds m^{-2}; mean of SC, C, SR and NT), corresponding to approximately 5.8% and 8.6 % of seed loss (Fig. 1). Soil inversion tillage without preceding stubble tillage (immediately after harvest) and no-till were the treatments which resulted in the lowest amount of seeds from seed loss entering the soil seed bank, though not significant. The soil seed bank was higher if there was immediate stubble tillable prior to primary tillage P or C; on average across the two varieties, SP resulted in 100 seeds m^{-2} more than P in the seed bank, corresponding to 3.5% of the seed loss; in the meanwhile SC resulted in 145 seeds m^{-2} more than C (1.6% of the seed loss).

3.3. CL OSR volunteers in 2014–2015

The factor variety had a significant effect on the number of flowering volunteers in the following crop WW 2014 (Fig. 2 A), and tillage had significant effects on the number of volunteers in WW and on the percentage of volunteers per tillage-specific seed loss (Fig. 2A and B). Across tillage treatments, medium dormancy variety 1 led to twice as many flowering volunteers as those of high dormancy variety 2 (1.9 vs. 0.88 plants m^{-2}), all without use of any herbicides, corresponding to 0.12% and 0.06% of seed loss (Fig. 2 B),

Table 5
Non-Clearfield oilseed rape (non-CL OSR) volunteers (plants m^{-2}) after harvesting on the stubble and the direct following crop winter wheat and Clearfield oilseed rape (CL OSR) in six tillage treatments; tillage in different levels of soil disturbance ranging from intensive soil inversion to no till (Table 2); experimental station Ihinger Hof, south-west Germany; no significant differences for results with the same letter ($P < 0.05$), compared only within the same counting time.

	SP	P	SC	C	SR	NT
Non-CL OSR volunteers in winter wheat 2011/2012 (plants m^{-2})						
Counting 1	10.00^{c}	436.25^{ab}	7.75^{c}	472.50^{a}	328.75^{ab}	307.50^{b}
Counting 2	0.00^{b}	0.00^{b}	0.00^{b}	0.50^{a}	0.00^{b}	0.25^{a}
Supposed non-CL OSR volunteers in CL OSR 2012/2013 (plants m^{-2})						
Counting 3	1.25^{ab}	0.00^{b}	1.25^{ab}	0.75^{ab}	3.50^{a}	0.25^{b}
Counting 4[(1)]	1.10^{b}	0.64^{b}	1.56^{ab}	1.19^{ab}	2.21^{a}	0.92^{b}

[(1)]Oilseed rape plants emerged between the rows of sown Clearfield oilseed rape; last herbicide application: Vantiga + Dash in September 2012 (Table 3).

Table 6
Table of significance for the effects of Clearfield oilseed rape (CL OSR) variety, long-term tillage systems, and their interactions on seed loss, seed bank, and proportion of seedbank/seed loss in the following crop winter wheat; experimental station Ihinger Hof, south-west Germany (during 2012–2014); two CL OSR varieties with medium dormancy (variety 1) and high dormancy (variety 2); tillage treatments see Table 2.

Effects	DF	Seed loss		Seed bank		Seedbank/seed loss	
		F-value	*P*	*F*-value	*P*	*F*-value	*P*
Variety (V)	1	0.05	0.8321	7.14	0.0155	5.25	0.0125
Tillage (T)	5	5.16	0.0012	0.79	0.5749	0.20	0.5318
$V \times T$	5	0.14	0.9821	0.49	0.7795	0.34	0.7450

Table 7
Seed loss (seeds m^{-2}) at harvest of Clearfield oilseed rape (CL OSR) and corresponding seed bank in the following crop winter wheat in spring 2014, as effect of variety (variety 1, medium dormancy; variety 2, high dormancy) and different tillage treatments (Table 2); experimental station Ihinger Hof, south-west Germany; different lowercase letters indicate significant differences between tillage treatments within the same variety at $P < 0.05$; capital letters are for comparison between two varieties at $P < 0.05$; values in parentheses are standard error of mean.

	SP	P	SC	C	SR	NT	Mean
Seed loss of CL OSR (seeds m^{-2})							
Variety 1	1967^{ab} (1104)	1271^{b} (1083)	2491^{a} (1066)	838^{c} (491)	1507^{b} (438)	1190^{b} (340)	1544^{A}
Variety 2	2291^{a} (1017)	1468^{b} (1049)	2010^{a} (586)	764^{c} (213)	1550^{b} (1140)	1191^{b} (667)	1546^{A}
Mean	2129^{a}	1370^{b}	2251^{a}	801^{c}	1529^{ab}	1191^{bc}	
Seed bank of CL OSR (seeds m^{-2}, 0–30 cm, first following crop WW, spring 2014)							
Variety 1	33^{a} (33)	34^{a} (33)	160^{a} (96)	31^{a}(31)	51^{a} (40)	37^{a} (36)	58^{B}
Variety 2	267^{a} (148)	67^{a} (52)	256^{a} (142)	93^{a} (65)	127^{a} (81)	73^{a} (55)	147^{A}
Mean	150^{a}	50^{a}	208^{a}	62^{a}	89^{a}	55^{a}	

Table 8
Number of oilseed rape volunteers per plot (mean of four replicates, plot size 9×50 m) with specific zygosity of the imidazolinone-tolerance genes PM_1 and PM_2 detected via real-time quantitative PCR. Sampling in winter wheat (spring 2014), after growing two Clearfield oilseed rape varieties with medium (variety 1) and high dormancy (variety 2) in the preceding year (2012/2013), and a non-CL variety in 2010/2011 in all plots; experimental station Ihinger Hof, south–west German; crops were grown in different long-term treatments of inversion and non-inversion tillage (see Table 2); samples taken from 20 volunteers per plot or fewer if fewer than 20 volunteers existed; wt: wildtype, ho: homozygous, he: heterozygous, und: undetermined (in PM_1 or PM_2).

Plant source	Tillage	No. of samples	Zygosity PM_1/PM_2									
			ho/ho	he/he	wt/wt	ho/he	he/ho	wt/ho	ho/und	wt/und	und/wt	und/ho
Variety 1	SP	16.5	15.5		0.3		0.3		0.3			0.3
	P	2.8	1.8		1							
	SC	19	17.8	0.3	0.5			0.3	0.3			
	C	15.8	15.3			0.3			0.3			
	SR	20	19.3	0.3	0.3				0.3			
	NT	20	20									
Variety 2	SP	17.8	15	1	0.5	0.3			0.5	0.3	0.3	
	P	3	1.5		1.5							
	SC	20	19.8		0.3							
	C	6.5	6.3		0.3							
	SR	12.3	11.5		0.8							
	NT	20	19.3	0.3		0.3			0.3			

respectively. Across varieties, treatments of NT and SC resulted in the highest number of flowering volunteers, corresponding to 0.40% and 0.01% of seed loss, while treatment P had the lowest number (0.001% of seed loss).

In the second following crop maize grown in 2015, there were no significant effects of the treatments on the number of volunteers. CL variety 1 still resulted in more volunteers than CL variety 2 in all tillage treatments except in NT (Fig. 3); the number of OSR volunteers ranged between zero in no-till and 1.4 plants 10 m^{-2} in SP.

3.4. DNA analysis of flowering volunteers 2014

Ten different gene combinations of PM_1 and PM_2 were detected in leaf discs of all the individual flowering volunteers in WW in spring 2014 (Table 8). Approximately 90% of sampled plants were

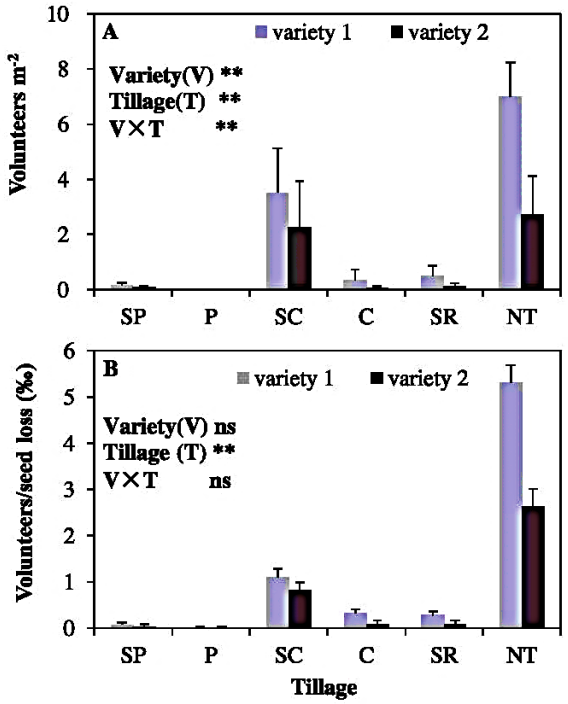

Fig. 2. Number of Clearfield oilseed rape flowering volunteers in the following crops winter wheat in 2014 (A), and their proportion (‰, promille, B) to seed loss at harvest in autumn 2013, as effect of variety (variety 1, medium dormancy; variety 2: high dormancy) and tillage (tillage treatments in Table 2); ** Significant at $P < 0.05$, ns: not significant; error bars indicate standard error of mean.

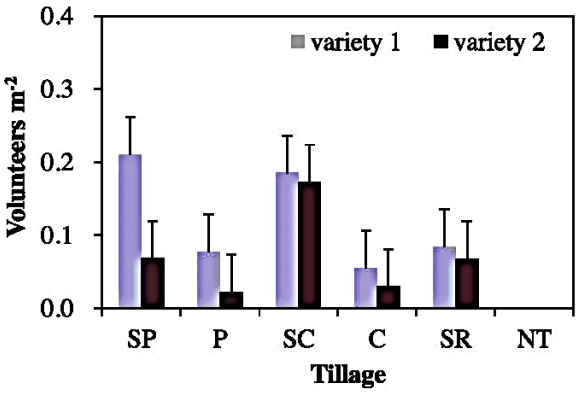

Fig. 3. Number of Clearfield oilseed rape volunteers in following crop maize 2015, as effect of variety (variety 1, medium dormancy; variety 2, high dormancy) and tillage; tillage treatments see Table 2; variety, tillage, and their interaction had no significant effects at $P < 0.05$; error bars indicate standard error of mean.

homozygous for PM_1 and PM_2 (PM_1/PM_2: ho/ho) in all the tillage treatments. In some tillage treatments i.e. SC and SR in combination with CL variety 1, and SP and NT with CL variety 2, 0.3 or 1.0 he/he plants (he: heterozygous) were detected. Divergent zygosity (he/ho, ho/he) was also identified with 0.3 plants in SP and C with variety 1 and in SP and NT with variety 2. For a few of the sampled plants of PM_1 or PM_2 zygosity such as ho/und, wt/und, und/wt, and und/ho could not be determined clearly because of failed PCR analysis (und: undetermined; wt: wild type). Pure wild type (wt/wt) volunteers were detected in small amounts in all tillage treatments except NT.

Table 9
Disposition to secondary seed dormancy (%) in seeds of Clearfield oilseed rape variety 1 and variety 2 in the generations F_1 (hybrid seeds), F_2 (harvested seeds), and F_3 (seeds produced by the F_2 volunteers in winter wheat at the experimental station Ihinger Hof, south–west Germany, 2014); different letters indicate significant differences at $P < 0.05$, compared only between varieties within the same generation; values in parentheses are standard error of mean.

Generation	Storage period (months)	Dormancy (%)	
		Variety 1	Variety 2
F_1	>8	0.8[b] (0.5)	92.4[a] (2.6)
F_2[(1)]	1	62.0[b] (1.2)	90.8[a] (1.2)
F_2[(2)]	4	6.8[b] (1.4)	63.0[a] (5.3)
F_3[(3)]	7	39.7[b] (9.5)	74.0[a] (6.6)

[(1)]Only seeds from tillage treatment SP, harvested by hand.
[(2)]Seeds in SP, harvested by combine harvester.
[(3)]Only seeds from tillage treatment SC.

3.5. Seed dormancy of CL varieties in F_1, F_2, and F_3 generations

Seed dormancy of CL OSR variety 1 was significantly lower than that of variety 2 over three generations, and the dormancy variation between generations of variety 1 was larger than that of variety 2 (Table 9). In the F_2 generation (seeds produced on F_1 plants), the scale of dormancy decline with storage time of variety 1 was remarkably faster than that of variety 2 (85.2% vs. 30.9%, proportion to initial dormancy of fresh seeds).

4. Discussion

4.1. Seed loss, seed bank, and volunteers as an effect of tillage and variety

The seed loss of CL OSR in this study was at a low level compared to that observed by other authors (Price et al., 1996; Weber et al., 2009), and much lower than the artificial seed inputs for experimental purposes by Gruber et al. (2004b). This fact and an uneven distribution of seeds after harvest could have masked effects of tillage and other experimental factors on the soil seed bank and volunteers, and made it difficult to detect clearly significant effects. Also, the significant effects of tillage treatments on seed loss were possibly due to maturity differences and seed yield differences among treatments (Alizadeh and Allameh, 2015). Although tillage treatments had no significant effects on the absolute numbers of seeds in the soil seed bank or on the proportion of seedbank/seedloss, the trend was similar to the findings of Gruber et al. (2004a) with stubble tillage after harvest leading to an increased soil seed bank in both inversion tillage and non-inversion tillage. Immediate incorporation of shed seeds by stubble tillage increased their chance to contact with soil conditions that can induce secondary dormancy, and therefore resulted in a larger soil seed bank than delayed tillage, similar to previous studies (Gruber et al., 2004a; Pekrun et al., 2006; Gruber et al., 2010). The accumulation of a soil seed bank is assumed to be site- and time-specific, depending on climatic conditions (Momoh et al., 2002; Gulden et al., 2004a). Precipitation before tillage was supposed to be a critical factor (Pekrun et al., 2006). Wet conditions, as they occurred in the present experiment during harvest time, may have resulted in an overall small soil seed bank.

Some seeds of two non-CL OSR varieties, Smart and Avatar from preceding growing in 2005 and 2011, respectively, were assumed to still exist in the seed bank in WW 2014 due to long-term seed persistence in the soil, but they could not be identified in the soil seed bank by the used methods. It has been shown that the deple-

tion of a seed bank takes several years after its establishment and depends on seed dormancy (Gruber et al., 2010), soil conditions (Pekrun et al., 2005), and soil disturbance (Lutman et al., 2003).

Genotypic differences in secondary dormancy significantly affected seed bank size in the present study; the variety with higher dormancy resulted in a larger seed bank than the variety with lower dormancy, in agreement with the findings of Gulden et al. (2003), Gruber et al. (2010) and Weber et al. (2014). Previous studies have indicated that secondary dormancy of the varieties is one crucial factor in determining seed bank size, but some other variety factors such as seed longevity through seed ingredients or attractiveness to predators may also contribute to the establishment and preservation of a soil seed bank.

The number of flowering CL OSR volunteers correlated positively with seed bank size in different tillage treatments, irrespective of the variety. However, the genotypic influence on volunteers was opposite to that of seed bank; variety 1 (medium dormancy) resulted in more volunteers than variety 2 (high dormancy), which was contrary to the results of Gruber et al. (2004c) and Weber et al. (2014). The main reasons for these observed differences from previous results may have been due to differences in seed loss and overall size of the soil seed bank. In the present study, the seed loss was much smaller than that of Gruber et al. (2004c) and of Weber et al. (2014), and there was a very small seed bank or no seed bank at all for the low dormancy variety in the study of Weber et al. (2014). Additionally, the differences may also be due to genotypic differences in germination, stress-resistance, or seed longevity of CL varieties 1 and 2. Third, dormancy release from the persisting seeds in the soil seed bank of the two CL varieties could have been different, with a faster release from dormancy in seeds of the medium dormancy variety. Varietal differences in the reaction of dormant oilseed rape seeds to abscisic acid (Gulden et al., 2004b) in terms of dormancy breaking and germination show that the effects observed in the field in our study can actually have a physiological background. Variety 1 was probably more prone to be released from dormancy in the field than variety 2, if the disposition to dormancy induction, which decreased during storage in variety 1 more than in seeds of variety 2 (Table 9), is considered as an indicator for the stability and maintenance of dormancy in the field. This would explain the higher number of volunteers in treatments with variety 1 though the soil seedbank was smaller here than that of variety 22 (58 vs. 147 seeds m^{-2}).

Though variety 1 led to higher numbers of volunteers in the first year after seed rain, its soil seed bank is considered short-lasting, while by contrast, variety 2 may lead to a long-lasting volunteer emergence because of the large soil seed bank. This was also shown in a 4.5-yr burial experiment of Gruber et al. (2010), 60% of initially buried seeds of high dormancy variety (91% dormancy) were still viable till the soil disturbance while only 8% of low dormancy variety (9% dormancy) survived. In this view, a very low dormancy variety (lower than variety 1) would be the best choice for volunteer control because of the smallest seed bank and a supposed fast dormancy release; in terms of CL volunteers, the majority of them would emerge in the first following crop, which is usually a cereal such as wheat, where crop competition is relatively high and where the broadleaf volunteers could be destroyed by herbicides with active ingredients alternative to imidazolinones. We hypothesize that a medium dormancy variety is the worst case because of a relatively large seed bank and fast dormancy release, which may result in many volunteers over a long time. A high dormancy variety would lead to a long-term volunteer emergence but with a small annual emergence rate. The release of OSR seeds from dormancy and its link to the initial dormancy level of the variety is a question for coming studies.

CL OSR volunteers in the present study were an effect of the tillage system (Counting 3 and 4, Table 5), with immediate incorporation of seeds after harvest increasing the number of volunteers even two years after seed rain, which is consistent with results of Gruber et al. (2004a), Gruber and Claupein (2006) and Pekrun et al. (2006). Moreover, inversion tillage resulted in 30 times fewer volunteers than non-inversion tillage in the first following year, most likely because (i) seeds were deeply buried and thus dormancy was probably maintained, and (ii) germinated seeds released from dormancy in deep soil would have died before emergence due to limited seed vigor. However, there was no difference on volunteer number between inversion and non-inversion tillage in the second year, probably as a consequence of seed return to the top soil by repeated soil inversion and seed depletion by germination, or by predation in the top soil layer of non-inversion tillage in the previous year. Overall, delayed inversion tillage without immediate stubble tillage in the first year and shallow non-inversion tillage in the subsequent years can be recommended after harvest of OSR in a crop sequence to avoid volunteers.

4.2. Genetic analysis of flowering OSR volunteers

Almost all flowering volunteers sampled in winter wheat in 2014 were homozygous for imidazolinone tolerance (ho/ho) and considered to be the offspring of CL OSR from the previous year. The small number of he/he plants and plants with other types of zygosity were probably from hybridization of non-CL and CL OSR due to the presence of non-CL volunteers in CL OSR in 2013 which survived the application of Vantiga or which emerged after the application, or due to seed impurities of the used CL varieties. Generally, the non-CL variety grown two years before was no longer visible in the volunteer OSR population; this genotype was probably not mixed to a great extent in the CL varieties, because its volunteers probably did not survive the Vantiaga applications, and did not spoil the seed and oil quality of the CL varieties. In non-CL OSR following in the next years in the crop rotation, however, CL volunteers for their part could not be killed by herbicides, would survive and would change the quality of the harvested OSR crop due to seed admixtures. The ratio of admixture by volunteers or outcrossing can be variable and long-lasting, and it is not only dependent on the duration and composition of the crop rotation (Messéan et al., 2007).

According to previous results, gene PM_2 conferred a much higher tolerance level than gene PM_1 (Tan et al., 2005); the tolerance of these two genes was unlinked and could be stacked, and the homozygous PM_1 and PM_2 (ho/ho) conferred the highest tolerance level. Given this, the volunteers with different gene combinations in this study were expected to have different tolerance levels, of which "ho/ho" for gene PM_1 and PM_2 conferred the highest tolerance, followed by "he/ho" and "ho/he" and then by "he/he", and last by "he/wt" (Krato et al., 2012; Krato and Petersen, 2012). Volunteers with genetic background other than ho/ho are supposed to have reduced levels of tolerance to imidazolinones, and thus a higher mortality than the ho/ho types when treated with herbicides according to the Clearfied® system, or with herbicides with similar active ingredients, as shown by Krato et al. (2012). This number of non-ho/ho volunteers, however, was very low so that most of the volunteers in the first following crop can be supposed to be CL-volunteers which could be only controlled by herbicides with other active ingredients. On the other hand, it can be concluded that the CL OSR crop in the year before was probably almost free from volunteers from preceding non-CL OSR.

5. Conclusion

The already known effect of small soil seed bank of OSR if the first post-harvest tillage is delayed can be fully confirmed and has

to be widely propagated in practical farming. An appropriate tillage strategy to reduce volunteers could be to bury seeds deeply by (delayed) deep inversion tillage in the first year, and then to perform tillage by (shallow) non-inversion tillage in following years. Growing low dormancy OSR varieties is a sound option to minimize the soil seed bank, in awareness that the majority of volunteers may mainly emerge in the first following crop.

Volunteers from CL OSR can be considered to be mainly homozygous in the tolerance genes and thus to show the same tolerance as the parental generation. After growing imidazolinone-tolerant oilseed rape with a low dormancy level, therefore, herbicides with active ingredients other than used in the Clearfield® system have to be applied particularly in the first following year, and crops with high competitive ability should be grown particularly in this year.

Acknowledgements

Many thanks to Ilona Weikert for excellent technical support. We also would like to thank the staff of the Experimental Station Ihinger Hof for the excellent field management and the support of China Scholarship Council (CSC).

References

Alizadeh, M.R., Allameh, A., 2015. Canola yield and yield components as affected by different tillage practices in paddy fields. Int. Agric. Sci. Nat. Res. 2 (3), 46–51.
Brimner, T.A., Gallivan, G.J., Stephenson, G.R., 2005. Influence of herbicide-resistant canola on the environmental impact of weed management. Pest Manag. Sci. 61 (1), 47–52.
Clements, D.R., Benott, D.L., Murphy, S.D., Swanton, C.J., 1996. Tillage effects on weed seed return and seedbank composition. Weed Sci. 44, 314–322.
Council Directive, 2002. Council Directive 2002/57/EC of 13 June 2002 on the marketing of seed of oil and fibre plants. Off. J. Eur. Commun. L 43, 74–97.
Gruber, S., Claupein, W., 2006. Effect of soil tillage intensity on seedbank dynamicsof oilseed rape compared with plastic pellets as reference material. J. Plant Dis. Prot. (XX), 273–280.
Gruber, S., Pekrun, C., Claupein, W., 2004a. Population dynamics of volunteer oilseed rape (*Brassica napus* L.) affected by tillage. Eur. J. Agron. 20 (4), 351–361.
Gruber, S., Pekrun, C., Claupein, W., 2004b. Seed persistence of oilseed rape (*Brassica napus*): variation in transgenic and conventionally bred cultivars. J. Agric. Sci. 142 (1), 29–40.
Gruber, S., Pekrun, C., Claupein, W., 2004c. Reducing oilseed rape (*Brassica napus*) volunteers by selecting varieties with low seed persistence. J. Plant Dis. Prot. (XIX), 151–159.
Gruber, S., Pekrun, C., Claupein, W., 2005. Life cycle and potential gene flow of volunteer oilseed rape in different tillage systems. Weed Res. 45 (2), 83–93.
Gruber, S., Bühler, A., Möhring, J., Claupein, W., 2010. Sleepers in the soil—vertical distribution by tillage and long-term survival of oilseed rape seeds compared with plastic pellets. Eur. J. Agron. 33 (2), 81–88.
Gulden, R.H., Shirtliffe, S.J., Thomas, A.G., 2003. Secondary seed dormancy prolongs persistence of volunteer canola in Western Canada. Weed Sci. 51 (6), 904–913.
Gulden, R.H., Thomas, A.G., Shirtliffe, S.J., 2004a. Secondary dormancy, temperature, and burial depth regulate seedbank dynamics in canola. Weed Sci. 52 (3), 382–388.
Gulden, R.H., Chiwocha, S., Abrams, S., McGregor, I., Kermode, A., Shirtliffe, S., 2004b. Response to abscisic acid application and hormone profiles in spring Brassica napus seed in relation to secondary dormancy. Can. J. Bot. 82 (11), 1618–1624.
Krato, C., Hartung, K., Petersen, J., 2012. Response of imidazolinone-tolerant and-susceptible volunteer oilseed rape (*Brassica napus* L.) to ALS inhibitors and alternative herbicides. Pest Manag. Sci. 68 (10), 1385–1392.
Krato, C., Petersen, J., 2012. Gene flow between imidazolinone-tolerant and-susceptible winter oilseed rape varieties. Weed Res. 52 (2), 187–196.
Laufer, C., Siebachmeyer, M., Gruber, S., Huang, S., Weber, E.A., Claupein, W., 2014. Against the current-Clearfield® oilseed rape in Germany. Julius-Kühn-Arch. 443, 720.
López-Granados, F., Lutman, P.J.W., 1998. Effect of environmental conditions on the dormancy and germination of volunteer oilseed rape seed (*Brassica napus*). Weed Sci. 46 (4), 419–423.
Lutman, P.J.W., Freeman, S.E., Pekrun, C., 2003. The long-term persistence of seeds of oilseed rape (*Brassica napus*) in arable fields. J. Agric. Sci. 141 (2), 231–240.
Lutman, P.J.W., Berry, K., Payne, R.W., Simpson, E., Sweet, J.B., Champion, G.T., May, M.J., Wightman, P., Walker, K., Lainsbury, M., 2005. Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (*Brassica napus*). Proc. R. Soc. Lond. B Biol. Sci. 272 (1575), 1909–1915.
Messéan, A., Sausse, C., Gasquez, J., Darmency, H., 2007. Occurrence of genetically modified oilseed rape seeds in the harvest of subsequent conventional oilseed rape over time. Eur. J. Agron. 27 (1), 115–122.
Momoh, E.J.J., Zhou, W.J., Kristiansson, B., 2002. Variation in the development of secondary dormancy in oilseed rape genotypes under conditions of stress. Weed Res. 42 (6), 446–455.
Pekrun, C., Lutman, P.J.W., Baeumer, K., 1997. Induction of secondary dormancy in rape seeds (*Brassica napus* L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. Eur. J. Agron. 6 (3–4), 245–255.
Pekrun, C., Lane, P.W., Lutman, P.J.W., 2005. Modelling seedbank dynamics of volunteer oilseed rape (*Brassica napus*). Agric. Syst. 84 (1), 1–20.
Pekrun, C., Lutman, P.J.W., Büchse, A., Albertini, A., Claupein, W., 2006. Reducing potential gene escape in time by appropriate post-harvest tillage—evidence from field experiments with oilseed rape at 10 sites in Europe. Eur. J. Agron. 25, 289–298.
Peltonen-Sainio, P., Pahkala, K., Mikkola, H., Jauhiainen, L., 2014. Seed loss and volunteer seedling establishment of rapeseed in the northernmost European conditions: potential for weed infestation and GM risks. Agric. Food Sci. 23 (4), 327–339.
Price, J.S., Hobson, R.N., Neale, M.A., Bruce, D.M., 1996. Seed losses in commercial harvesting of oilseed rape. J. Agric. Eng. Res. 65, 183–191.
Tan, S., Evans, R.R., Dahmer, M.L., Singh, B.K., Shaner, D.L., 2005. Imidazolinone-tolerant crops: history, current status and future. Pest Manag. Sci. 61 (3), 246–257.
Weber, E.A., Frick, K., Gruber, S., Claupein, W., 2010. Research and development towards a laboratory method for testing the genotypic predisposition of oilseed rape (*Brassica napus* L.) to secondary dormancy. Seed Sci. Technol. 38, 298–310.
Weber, A., Gruber, S., Claupein, W., 2014. Emergence and performance of volunteer oilseed rape (*Brassica napus*) in different crops. Eur. J. Agron. 60, 33–40.
Zhu, Y.M., Li, Y.D., Colbach, N., Ma, K.P., Wei, W., Mi, X.C., 2012. Seed losses at harvest and seed persistence of oilseed rape (*Brassica napus*) in different cultural conditions in Chinese farming systems. Weed Res. 52 (4), 317–326.

6 General discussion

In the previous chapters (I-III), various aspects of seed dormancy of CL OSR and tillage effects on soil seed bank and volunteers in the following crops after OSR have been evaluated. The primary results are presented in the above three scientific articles, followed by an individually detailed discussion at the end of each article. In the general discussion, the main results from chapter I-III are summarized with the aim to examine the initially proposed hypotheses and finally to deal with the future research in this field.

The present chapter also focused on the discussion of obtained results in a broader context of scientific literatures; for instance, overall evaluation of **seed dormancy & dormancy formation** during seed development of OSR, **maternal environment** effects and **post-harvest environment** effects on dormancy dynamics, as well as suitable **cropping systems** to reduce soil seed bank and volunteers of OSR. Soil seed bank dynamics and volunteer emergence in a long run as an important aspect of gene dispersal in time were further discussed. In particular, dormancy release over time when seeds are buried in the soil is integrated into this chapter. An integrated management strategy of OSR volunteers was discussed and assessed based on previous and present results.

6.1 Seed dormancy

6.1.1 Varietal effects

This study confirmed the variation of predisposition to secondary dormancy (potential secondary dormancy from laboratory analyses; Weber et al., 2010) of mature OSR seeds between varieties shown by Gulden et al. (2004a, 2009), Gruber et al., (2004a), Weber et al. (2013) as well as the dormancy variation within populations of the same variety (Weber et al., 2013). Due to the dormancy variation, seed persistence of OSR in the soil seed bank varies considerably, ranging from a few months to over 10 years, depending on variety (Lutman et al., 2003). Correspondingly, varieties with a high dormancy level can give rise in occurrence of volunteer OSR for a longer period (Messéan et al., 2007; D'Hertefeldt et al., 2008).

The genetic background to secondary seed dormancy is relatively strong (Schatzki, et al., 2013a), indicating that selecting or breeding for low dormancy OSR variety is feasible. It is estimated that sowing low dormancy OSR varieties is an effective method to control OSR volunteers in the following crops after OSR (Gruber and Claupein, 2007; Weber et

al., 2014); hence it is encouraged to classify the current varieties into different dormancy groups (low, medium and high) according to their dormancy levels in agricultural practice (Gruber et al., 2009).

In CL OSR, mature seeds showed similar dormancy variation between or within varieties to that of non-CL OSR (Chapter I and II). The current CL OSR varieties or lines (genotypes) can also be classified into low, medium, and high dormancy groups; out of the 15 tested CL genotypes, nine genotypes can be considered as low dormancy (<30%, dormancy level). The inheritability of potential secondary dormancy from F_1 to F_2 generations is stable. This suggests that low dormancy CL OSR variety can be achieved by selection or breeding method, providing a strategy from genotypic perspective to control CL OSR volunteers.

The contribution of variety to secondary dormancy of OSR has been calculated in a range of 44-82%, probably related to seed size (Gulden et al., 2004a) and stage of maturation of the seeds (Haile and Shirtliffe, 2014). And this is likely to be associated with gene expression (Fei et al., 2007, 2009; Schatzki et al., 2013b). Much more genes can be up-regulated in high dormancy OSR varieties than in low dormancy varieties when seeds are exposed to specific osmotic conditions. These genes are supposed to be involved in ABA biosynthesis (Fei et al., 2007, 2009). The *DELAY OF GERMINATION 1* (*DOG1*) gene, which is essential for seed dormancy in *Arabidopsis thaliana* is likely to regulate induction of secondary dormancy in OSR, named *BnaDOG1* gene (Né et al., 2015). *DOG1* is dependent on ABA (Nonogaki, 2015). The difference between varieties in induction of secondary dormancy starts to appear at mid-stage of seed development and becomes more apparent over time (Chapter I). This could be explained by the difference between varieties in gene expression during the transition from full-size embryo to maturity (Fei et al., 2007). Yet, the physiological role of these genes in dormancy induction is far from being understood.

Compared to secondary dormancy, primary seed dormancy is easier to be understood. The initial primary dormancy level is high probably resulting from an under-developed embryo at early seed development (Bewley, 1997), and remains at low level at maturity (Momoh et al., 2002; Gulden et al., 2004a), associated with seed ABA content in OSR (Finkelstein et al., 1985; Nambara et al., 2010). Two QTLs were detected for primary seed dormancy of OSR (Schatzki et al., 2013b). Several primary dormancy-related genes such as *DOG1*, *ABA deficient1* (*ABA1*), and *ABA INSENSITIVE 3, 4, 5* (*ABI3*) in *Arabidopsis thaliana*

have been discovered (Angelovici et al., 2010; Graeber et al., 2012). However, evidences in genetic and molecular control of primary dormancy in OSR are still absent.

Primary dormancy of OSR is supposed to be a small contributor to the development of soil seed bank due to low dormancy level in mature seeds (Momoh et al., 2002; Gulden et al., 2003). Considering the large harvest loss (up to 10 000 seeds m^{-2}; Lutman et al., 2005), its contribution cannot however be negligible, e.g. a mean primary dormancy level of 3% across 20 OSR varieties at normal harvest was detected (Gruber et al., 2004a), corresponding to up to 300 dormant seeds m^{-2} located on the soil surface. In particular, primary dormancy in fresh seeds of most varieties and in mature seeds of some varieties is high (Haile and Shirtliffe, 2014), which was also found in CL OSR in the present study. With this in mind, a hailstorm or a strong wind during late seed development of OSR probably could result in a large soil seed bank as a consequence of primary seed dormancy.

To date, no special studies have focused on varietal differences in the release of OSR seeds from dormancy, which is closely associated with the occurrence of volunteers in the long run, especially after the development of soil seed bank. The results in Chapter III of this thesis indicate that medium-dormant OSR variety can result in more volunteers than high-dormant variety. This effect is probably attributed to the difference in the speed of dormancy release. The speed of release from dormancy could be independent from the level of dormancy but linked with any traits of the seed such as oil content or seed size, or it could be linked to the dormancy level. The evidence in dormancy release of OSR has still been particularly lacking. This study states the hypothesis that low dormancy varieties would be released from dormancy more rapidly than high dormancy varieties. The study, however, compared only two varieties, so that the hypothesis has to be tested in more detailed, following studies with varieties different in dormancy levels and in other seed traits.

If the OSR varieties available on the market could be classified into different groups based on the speed of secondary dormancy release (i.e. slow, medium, and fast), another variety-related approach to control volunteers would be possible, apart from breeding low-dormancy varieties. Therefore, dormancy release in OSR needs to become a goal in the next research step.

6.1.2 Harvest time

Harvest time (or stage of seed maturation at harvest) effects on primary and secondary dormancy are significant in OSR (Haile and Shirtliffe, 2014). Before full-size stage of seed embryo, primary dormancy was high and potential secondary dormancy was nearly zero (Chapter I); but after that stage, primary dormancy declined to a relatively low level and potential secondary dormancy increased over time. Evidence in the relationship between primary and potential secondary dormancy during seed development of OSR is still not enough. Probably, there is a link at early seed development based on the significant correlation between them, but the link became weak with further seed development. It seemed that freshly harvested seeds with high primary dormancy can be induced secondary dormancy more easily in OSR (Gruber et al., 2004a; Schatzki et al., 2013b), which was also found and discussed in *Arabidopsis thaliana* by Auge et al. (2015). Moreover, the results in Chapter I also indicate that seed dormancy of OSR is dependent on some processes essential for seed viability and germination during seed development. These processes are estimated to associate with endogenous ABA biosynthesis (Finkelstein et al., 1985; Bewley, 1997) and embryo sensitivity to ABA (Hilhorst, 1995; Juricic et al., 1995). In agricultural practice, the dormancy-related processes during the late seed development are supposed to be more important to determine an appropriate harvest time. Therefore, full seed maturity is necessary for combine harvesting if no primary dormancy is wanted, in contrast to windrowing which would allow the seeds to after-ripen. This would mainly apply for harvests of seeds to be used for immediately sowing the next OSR crop.

It is known that primary dormancy and potential secondary dormancy of OSR decreases over time during seed storage (Gruber et al., 2004a; Gulden et al., 2004a). In Chapter I, it was shown that the decline of potential secondary dormancy already appeared before full seed maturation. The highest peak in potential secondary dormancy, which seemed to occur 60-70 days after flowering depending on variety, must be avoided in harvest. Besides, evidence from Gruber et al. (2004a) showed that primary seed dormancy of OSR vanished and potential secondary dormancy decreased by about nine percent points after six months of seed storage at room temperature (18-25℃). This suggested that seeds being stored for one year and then sowed would result in a lesser dormancy induction at sowing.

Compared to combine harvesting, the operation time of windrowing, is supposed not to have important effects on seed dormancy. The seed dormancy dynamics of OSR during the

period from windrowing to seed threshing is still unclear. In this period, the supply of seeds with assimilates and water by the mother plant is interrupted and maturation is fostered after windrowing (Irvine and Lafond, 2010). There are currently not many results available about dormancy dynamics under the conditions of interrupted ripening, such as during windrowing, or if seeds drop onto the soil through hailstorm.

Harvesting at the optimum stage can give lower seed losses; delayed harvesting would increase the risk of seed losses from the standing crop by natural opening of the pods (Thomas et al., 1991). It is more difficult to determine the optimal harvest time for winter OSR compared with spring OSR because of the longer and less uniform period of seed ripening (Thomas et al., 1991; Price et al., 1996). This is also affected by environmental conditions surrounding mother plants, e.g. the drier the conditions the larger the harvest losses, associated with hot and windy weather. Therefore, avoiding harvest at a hot temperature with a low air moisture and during windy conditions is necessary (Thomas et al., 1991). Seed losses of OSR resulting from delayed harvesting or improper harvesting conditions seem to be greater than those from harvesting methods (Lutman, 2003; Pekrun et al., 2003). Advance in breeding pod shatter resistant varieties is encouraging, but it is still an ambitious goal (Morgan et al., 2000; Hossain et al., 2011).

Seeds from seed propagation, used for sowing the next OSR crop, may also result in a soil seed bank if they are seeded shortly after harvest due to both high primary dormancy and potential secondary dormancy. Besides, seed dispersal by harvesting machine or seed spillage during transportation can cause a soil seed bank at feral area such as road sides, resulting in feral OSR populations (Garnier et al., 2008; Pivard et al., 2008a, b). In particular, seeds lost during hailstorms, which frequently occur in Europe during seed development of OSR, are supposed to have a high primary dormancy level (Chapter I; Haile and Shirtliffe, 2014), directly giving rise a soil seed bank without induction of secondary dormancy.

6.1.3 Maternal environment and post-harvest environment

The predisposition to secondary dormancy of OSR varies between locations and years (Momoh et al., 2002; Gruber et al., 2009; Weber et al., 2013), which is partly attributed to the difference in maternal environment. Seed dormancy levels for many plant species have been found to differ between habitats related to latitude and elevation (Baskin and Baskin, 1998), reflecting plant adaptation to different maternal environments (Alonso-Blanco et

al., 2009). Precisely, precipitation and temperature in seed ripening are involved in the influence of location or year on seed dormancy dynamics. For instance, high temperature and drought can result in low primary seed dormancy in wheat (Garello and Le Page-Degivry, 1999; Wright et al., 1999). Maternal environmental effects on seed dormancy are limited, but its interaction with variety is obvious in *Arabidopsis thaliana* (Postma and Ågren, 2015). It has been estimated that maternal environmental effects can influence the expression of genetic variation (i.e., *DOG1*) for seed dormancy in *Arabidopsis thaliana* (Donohue, 2009). There also appears to be an influence of maternal environment on secondary seed dormancy in OSR (Gulden et al., 2004a; Schatzki et al., 2013b), but the direct influencing factors were not mentioned. **Chapter II** of this thesis highlighted the importance of precipitation and temperature over seed ripening on the dynamics of potential secondary dormancy in OSR for the first time; locations with low precipitation and high temperature during maturation could lead to a lower level of potential secondary dormancy in OSR seeds. This indicates that it is possible to select suitable location for breeding low dormancy varieties.

It is well known that **post-harvest environmental conditions** play a critical role in the development and dynamics of the soil seed bank of OSR (Pekrun et al., 2005, 2006). During the period between harvest of OSR and the subsequent, first tillage operation, three processes in seed dormancy can be defined:

1. Decline of primary dormancy if primary dormancy exists in mature seeds; this period probably cannot last long time (probably a few weeks) due to the low dormancy level, depending on variety and storage conditions.
2. Decline of the predisposition to secondary dormancy which is supposed to last longer (probably several months) than the decline of primary dormancy probably due to high potential secondary dormancy level.
3. Induction of secondary dormancy, which probably takes place in the first two weeks after the seeds were exposed to the soil environment.

Explicit experiments about secondary seed dormancy induction on soil surface in natural environment for OSR was not found. In one pot experiment of Gruber et al. (2010), about 50% of artificial input of OSR seeds falling dormancy at the soil surface were ever found. One of our field trials aiming at investigating effects of timing and depth of stubble tillage post-harvest on soil seed bank and volunteers of OSR, presented no or very small soil seed

bank and no volunteers in the following crop winter wheat (data not shown). This field trial was conducted at two sites (Ihinger Hof experimental station of the University of Hohenheim and Hohenheim) in two experimental approaches 2012-2013 and 2013-2014. By contrast, a similar field trial about effects of delayed tillage with or without stubble tillage was also performed at Ihinger Hof experimental station but in 2001-2002 and 2002-2003 by Gruber et al. (2004b, c, 2005), in which a large soil seed bank and a high number of volunteers were detected. The biggest difference between these trials was the higher precipitation after harvest of OSR in the years 2012 and 2013 which could explain the difference in soil seed bank and volunteers from the following aspects. First, due to the usually low primary dormancy in harvested OSR seeds, most of the lost seeds are not or slightly dormant at harvest, which can be triggered into germination by precipitations. Second, the hydrothermal values (temperature of 3.8℃and water potential of -1.4 MPa) for germination of OSR are quite low (Soltani et al., 2013), so soil moisture even resulting from a slight rain is supposed to give rise to germination for the lost seeds in a short period. These might be also the reasons for the absence of CL OSR volunteers in the first following crops (most of them were winter wheat) at 20 out of 41 monitoring sites nearly across the whole of Germany in 2012-2013 (Laufer et al., 2014).

The emerged seedlings in the first autumn after harvest of OSR can reach up to 70% of all seeds dropped onto the soil (Pekrun, et al., 2006). The autumn emergence is obviously not closely correlated with soil seed bank size in next spring after OSR (Pekrun, et al., 2006; Weber et al., 2014), suggesting that post-harvest environmental conditions inducing dormancy is more important than conditions that trigger seed germination. The quantitative effects of each seed fates such as emergence, seed death, predation, dormancy and decay have not yet been fully understood.

6.2 Tillage effects on the soil seed bank

Conventional tillage (inversion tillage; mouldboard ploughing), conservation tillage (non-inversion tillage, i.e., by chisel ploughing, rototiller), with or without preceding shallow stubble tillage or no-tillage are main categories of soil cultivation. These tillage operations performed after harvest of OSR can affect number and distribution of OSR seeds in the soil to a great extent (Pekrun et al., 1998; Gruber et al., 2007, 2010). Regarding the size of the OSR **soil seed bank**, previous studies did not show clear and consistent results for the mode of tillage. For instance, chisel ploughing resulted in a larger soil seed bank of OSR compared to mouldboard ploughing in the study of Gruber et al. (2004b). No significant

difference between inversion ploughing and non-inversion cultivation was detected in the study of Pekrun et al. (2006), similar as shown in **Chapter III** of this thesis. For weed soil seed banks, there were no significant difference on the size of the seed bank either (Plaza et al., 2011; Ruisi et al., 2015), but in a long run or in organic farming, non-inversion cultivation generally results in larger soil seed banks (Cardina et al., 2002; Sosnoskie et al., 2006; Gruber and Claupein, 2009). The inconsistent results are likely related to post-harvest environmental conditions (Gulden et al., 2004a, c) or soil types (Swanton et al., 2000; Gruber et al., 2014). Nevertheless, no-till in general results in a smaller soil seed bank size of OSR than other modes of tillage.

In contrast to seed bank size, effects of tillage modes on **seed distribution** are more consistent; mouldboard ploughing can bury more seeds in the deep soil, whereas chisel ploughing mixes seeds within the tilled soil layer, and no-till leaves most of lost seeds on the soil surface (Colbach et al., 2000; Swanton et al., 2000; Gruber et al., 2010). In the second following year, deeply buried seeds, however, can be turned back into upper soil layer by repeat mouldboard ploughing (Colbach et al., 2000; Momoh et al., 2006). The soil burial depth might affect secondary seed dormancy induction of OSR, in interaction with external environmental conditions (Gulden et al., 2004c; Soltani et al., 2013). Under pot experimental conditions in greenhouse, more dormant OSR seeds were found at both soil surface and deep soil layers (<12cm) than in the middle soil of 1-7 cm (Gruber et al., 2010). Dryness at the soil surface, or darkness and low oxygen in the deep soil probably contribute to dormancy induction and large soil seed banks. Under field conditions, more OSR seeds seem to be induced into dormancy in the soil below top soil layer, which can explain the smaller soil seed bank frequently found in no-till treatment (Pekrun et al., 1998; Gruber et al., 2004b, 2005, 2010). In some weed species, it is estimated that seed dormancy increases with burial depth (Benvenuti, et al., 2001; Sester et al., 2007; Gruber and Claupein, 2009). Compared with dormancy induction, the suppressed effects of burial depth on seedling pre-emergence growth of non-dormant OSR seeds tend to be more obvious (Lutman, 1993; Gulden et al., 2004c; Pekrun et al., 2005). If the burial depth is over 10 cm, the seedlings are assumed not to emerge due to the limited seed reserves (Gruber et al., 2011; Soltani et al., 2013).

A consensus in **effects of operation time** of post-harvest tillage on soil seed bank of OSR has been achieved. i.e. delayed soil disturbance after harvest can reduce soil seed bank size even by shallow stubble tillage (Lutman et al., 2003; Gruber et al., 2004b; Pekrun et al.,

2006), which was further confirmed and thoroughly discussed in Chapter III. However, when seeds are exposure to dry conditions, which is the most effective inducing factor for secondary dormancy in OSR, some exceptions can be found. More dormant seeds were, for instance, found in two-week delayed tillage treatment than in immediate tillage post-harvest, but only in one out of ten experimental sites of Pekrun et al. (2006). Differences in loss of primary dormancy and in induction of secondary dormancy between seeds on the soil surface and seeds buried in the soil can partly explain the underlying mechanisms. Explicit studies of the mechanisms under field conditions were not available. Hence, more burial experiments with fresh OSR seeds which have both primary dormancy and potential secondary dormancy under controlled environments are needed. Generally, it is recommended to keep stubbles undisturbed for several weeks after harvest of OSR with the aim at reducing both soil seed bank size and volunteer weed problems in the subsequent crops (Gruber et al., 2004b; Pekrun et al., 2006).

6.3 Soil seed bank dynamics and volunteer emergence

Once a soil seed bank is created, its size will decrease exponentially with time and with burial depth as a consequence of dormancy loss, germination and natural death in weeds or OSR (Squire et al., 1997a; Sester et al., 2006; Soltani et al., 2013). The seed number declines rapidly in a few months after development of soil seed bank, and then slows down during the subsequent seasons, depending on soil environment and variety (Gulden et al., 2003; Lutman et al., 2003, 2005; Gruber et al., 2004a, 2010).

Seasonal soil environment is assumed to regulate the dormancy status of buried seeds in some plant species including OSR (Batlla and Benech-Arnold, 2004; Pekrun et al., 2005; Sester et al., 2007; Colbach et al., 2008). In some summer annual weeds, seed dormancy of seeds in the soil seed bank increases in summer and autumn and decreases in winter (Batlla and Benech-Arnold, 2004; Sester et al., 2007). Colbach et al. (2008) proposed that seed dormancy level in OSR could be expressed in sinusoidal wave as a consequence of seasonal soil moisture, temperature and burial depth. There is supposed to be a threshold temperature, below which seed dormancy will decline faster in *Polygonum aviculare* L. (Batlla and Benech-Arnold, 2004), in interaction with light and nitrate (Çetinbaş and Koyuncu, 2006; Finch-Savage et al., 2007). In OSR, low temperature and potassium nitrate (KNO_3) are supposed to force loss of secondary dormancy (ISTA 2007), and alternating light and temperature is proved to be the most effective way to break secondary seed dormancy (Weber et al., 2010). Nevertheless, the threshold temperature for dormancy loss

in OSR and quantified contributions of soil climates are still quite far from being clearly defined.

Regarding variety influence on seed persistence, high dormancy varieties can result in a longer persistence than medium and low dormancy varieties in OSR, due to larger soil seed bank (Gulden et al., 2003; Gruber et al., 2010) or to slower dormancy release. However, the evidence in dormancy release between OSR varieties is still limited.

After release of secondary dormancy in buried seeds of OSR, their emergence seems to be strongly dependent on burial depth, hydrothermal time, seed reserves and soil structure. Non-dormant seeds located in a top soil layer (e.g. 0-5cm) more likely emerge as volunteers in the following crop (Pekrun et al., 2005; Gruber and Claupein, 2006; Gruber et al., 2010; Soltani et al., 2013) depending on soil moisture and temperature (Colbach et al., 2008). In deeper soil layer, the emergence rate will decrease exponentially with burial depth (Gruber et al., 2004a; Gulden et al., 2004c; Sester et al., 2007), associated with soil texture (Swanton et al., 2000; Dürr et al., 2001).

After emergence, plant disease, insects, herbicides, crop competition, and their interactions can impact OSR volunteers and reduce their number and/or impair their performance and reproductive capacity to some extent. This thesis included results from experiments without any chemical control, and thus showed the worst case scenarios where no chemical and mechanical weed control either was applied or was efficient. The situation in the field would be most likely different from this situation. There are herbicides in selection for broadleaf weeds including OSR volunteers. Even if the volunteers would be tolerant to some active ingredients (as CL OSR is), the efficacy of herbicides would probably not be always 100% but rather 70-90%, depending on varieties and application time (Krato et al., 2012).

However, there might be still some OSR volunteers surviving until the harvest of subsequent crops, especially in rapeseed crops. In previous study of Gruber and Claupein (2007), seed production of volunteer OSR in cereal crops was about 10% of sown OSR, and about one seed m^{-2} could replenish the soil seed bank; in subsequently sown OSR crop, however, volunteer seed production can reach up to 100% and the same number of seeds as the sown crop could return to the seed bank.

6.4 Management of CL OSR volunteers

Seed dormancy characteristics, soil seed bank dynamics, and volunteer occurrence of CL OSR are similar to that of non-CL OSR, based on the comparison between results in chapter I-III and previous results. Although gene segregation in herbicide tolerance exists in CL OSR volunteers, most of the volunteers (>80%) are still homozygous in both *PM₁* and *PM₂* genes (Chapter III). This indicated that the highest tolerance to imidazolinone herbicides was also existent in the volunteers. Therefore, suitable strategies to control volunteer CL OSR are listed and assessed in the following:

1. **Harvest scenarios:** there are two harvest scenarios, early harvesting with a high seed dormancy (both primary dormancy and potential secondary dormancy) but low harvest losses, and late harvesting with high risk of seed losses (Thomas et al., 1991) but low seed dormancy (particularly primary dormancy). Determination of the optimal harvest time is quite complex because of different ripening of seeds depending on pod positions on the plants (Diepenbrock and Geisler, 1979). Apart from seed dormancy and seed losses, early harvesting can reduce seed yield and seed quality (Rathke et al., 2006). Overall, as recommended by Haile and Shirtliffe (2014), OSR should not be harvested earlier than 60% seed color change for the harvesting method of windrowing. For the combine harvesting, avoiding harvest at the stage, at which OSR seeds have the highest potential secondary dormancy, is necessary, along with avoidance of unsuitable harvesting weather conditions such as high temperature, low air humidity and wind.
2. **The use of low dormancy varieties:** the ideal varieties with low potential for OSR volunteer occurrence would have no primary dormancy, very low or no potential for secondary dormancy, and would be released from dormancy very fast. If release from dormancy would occur within the first year, with usually cereals as first following crop after OSR, the volunteers could be controlled by supplemental herbicides or by choosing crop densities or varieties of cereals which compete with OSR volunteers. High dormancy varieties, in contrast, are supposed to cause long-term seed persistence, large seed banks and volunteers in subsequent crops several years after the harvest of OSR, especially in another sown OSR crop where no chemical control is possible.
3. **Tillage modes and tillage operation timing:** considering the difference between modes of tillage in the volunteer emergence (Pekrun et al., 1998; Gruber et al., 2004b), combination of tillage modes in different following years after OSR can probably control OSR volunteers in a long run. As shown in Chapter III, inversion tillage by

mouldboard ploughing resulted in very few or no volunteers in the first following year, but resulted in relatively more volunteers in the second year due to soil return to shallow soil layer. Non-inversion tillage or no-till led to more volunteers in the first year, and to fewer in the second year due to soil seed bank depletion. Besides, a time break of 3-4 weeks between harvest of OSR and the first following tillage can result in a smaller soil seed bank compared to immediate tillage. Therefore, performing delayed deep inversion tillage in the first year after OSR and shallow non-inversion tillage in subsequent years is recommended in the control of OSR volunteers.

4. **Alternative herbicides** with different active ingredients other than ALS- inhibiting herbicides (e.g. imidazolinones and sulfonylureas) should be considered in the control of CL OSR volunteers, especially for homozygous imidazolinone-tolerant (HOM_IT) plants. Herbicide florasulam, one of Triazolopyrimidine sulfonanilide-based herbicide, was considered as the most effective herbicide treatment in the study of Krato et al. (2012). Its efficacy can reach up to 100% for heterozygous imidazolinone-tolerant (HET_IT) volunteers and to about 90% for HOM_IT volunteers under different environmental conditions. Based on the field survey in chapter III of this thesis, across six tillage treatments, 4.04 and 1.77 HOM_IT volunteers m^{-2} and 0.06 and 0.07 HET_IT volunteers m^{-2} were found for medium and high dormancy CL OSR varieties in the first following spring (data not shown), respectively. After florasulam application, 0.41 and 0.18 HOM_IT volunteers m^{-2} are supposed to survive for the two CL OSR varieties used, which are still high numbers of OSR volunteer in the following crops after OSR (Gruber and Claupein, 2007; Messéan et al., 2007). If imidazolinones or sulfonylureas herbicides were applied, more volunteer survivors could be detected (Krato et al., 2012; Krato and Petersen, 2012; Schwabe et al., 2016). Efficacy of flupyrsulfuron (one sulfonylureas herbicide), for instance, was 95% and 50% in the control of HET_IT and HOM_IT CL OSR volunteers (Krato et al., 2012), and correspondingly, 2.07 and 0.95 CL OSR volunteers (sum of HET_IT and HOM_IT plants) of medium and high dormancy CL varieties used in Chapter III of this thesis would survive the herbicide application in the first following crop winter wheat.

Besides, due to the difference in burial depth and in dormancy release between OSR seeds, the volunteer emergence is supposed to be uneven. In this context, the efficacy of herbicides to the volunteers emerging after herbicide application will be compromised. Hence, cultural practices, particularly tillage regimes, are recommended to be used conscientiously in the first place before sowing the subsequent crop to reduce

OSR volunteers to the lowest possible level (Krato et al., 2012).

5. **Crop rotation:** the choice of the following crops after CL OSR is supposed to be crucial in the control of OSR volunteers (Colbach et al., 2001; Devos et al., 2004). OSR crop is mainly grown in one out of three or four years in rotations with a high proportion of cereals in Germany (Rathke et al., 2005; Rathke and Diepenbrock, 2006). In general, cereals are the first following crops after OSR in crop rotations in Germany such as winter OSR (WOSR)-winter wheat (WW)-winter barley (WB), WOSR-WW, and WOSR-WW-WW-soybean (SB). Control of OSR volunteers in cereal crops is easy and inexpensive because of various herbicide options.

   Crop competition is also a contributor to the control of weeds or crop volunteers, depending on plant density and morphological structures (Colbach et al., 2001; Rajcan and Swanton, 2001; Weiner et al., 2001). Cereal crops with high plant density and a high competitive morphology (such as planophile leaves and a fast development) which can suppress weeds or volunteers should be considered. Increasing the length of the rotation can reduce the number of OSR volunteers and their adverse impacts into the following oilseed rape crop.

As another issue, herbicide-tolerant genes can be dispersed from herbicide-tolerant OSR to non-herbicide tolerant OSR with pollen flow (Devos et al., 2004; Hüsken and Dietz-Pfeilstetter, 2007; Krato and Petersen, 2012) due to the high cross-pollination (Becker et al., 1992; Cuthbert and McVetty, 2001). Most of the outcrossed seeds (more than 80%) of CL OSR, which mainly occurred at the nearest area of pollen acceptor fields to CL OSR fields, were heterozygous in both *PM1* and *PM2* genes (Krato and Petersen, 2012). Even at 45 m from CL OSR field, HET_IT seeds also can be found in non-CL field. If these seeds fall on the soil surface during harvest and become a component of the soil seed bank, HET_IT OSR volunteers are supposed to appear in the following crops, increasing the difficulty of weed control in adjacent fields. Therefore, suitable cultural practices for limiting pollen-mediated gene flow of GM OSR (Ingram, 2000; Staniland et al., 2000; Damgaard and Kjellsson, 2005) probably can be applied to CL OSR, such as isolation distance between CL and non-CL fields, buffer zone in non-CL fields, or separately harvesting border area of non-CL fields at early seed stage to reduce harvest loss.

In the monitoring of Laufer et al. (2014), nearly half of the monitored sites (20 out of 40 sites all over Germany) had no soil seed bank and volunteers of CL OSR at all in the first

following crops after OSR, most of which were winter wheat. The farmers at these sites must have made some things correctly, such as in the choice of post-harvest tillage and herbicides. In addition, weather conditions, especially between harvest of OSR and first subsequent tillage was not sufficient for dormancy induction, probably associated with a high precipitation in this period. Similarly, nearly neither OSR soil seed banks nor volunteers were detected in one of our field trials even with a huge artificial seed input of 5000-20,000 seeds m^{-2} of high dormancy OSR variety (91% induced secondary dormancy level) in 2012-2015 at Hohenheim (data not shown). This is assumed to be attributed to the high precipitations in the first autumn after harvest of OSR. All in all, farmers' practice is obviously often suitable to control OSR volunteers even without knowing the details in the volunteer control. Combining with the recommended cultural practices in this study, CL OSR volunteers are supposed to be reduced to a very low level.

Considering the advantages of the Clearfield® production system in weed control (post-emergence, working flexibility, and broad-spectrum), CL OSR will probably be widely accepted by famers in Europe. This will increase the debate on the adverse impacts of CL OSR volunteers on the following crops. Based on the current knowledge achieved in this study (Table 1) and previous studies (Krato et al., 2012; Krato and Petersen, 2012; Laufer et al., 2014), the CL volunteers can be well controlled by mechanical and chemical methods pre- and post-sowing of next crops. However, as the herbicide tolerance (i.e. ALS-inhibitors) and particularly long-term seed persistence if a large soil seed bank is developed, it is quite difficult to control these CL volunteers by herbicides in crop rotations with non-CL OSR in a long run. Therefore, it is particularly important to reduce the potential development of soil seed bank after harvest of OSR. To our knowledge, an integrated strategy should be considered, including variety-dependent dormancy (low dormancy), harvest date (relative late harvest to avoid high seed dormancy, depending on harvesting method), and post-harvest tillage (delayed and deep burial). Also, proper choice of competitive following crops (planophile leaves and a fast development) and alternative herbicides (non-ALS inhibitors) to suppress CL volunteers. After harvest of the first following crops, shallow non-inversion tillage should be used for several years to keep CL seeds buried deeply in the soil.

To date, there are still lots of knowledge gaps in the life cycle of OSR volunteers that will be focused in next research steps (Table 1), e.g. (i) quantification of post-harvest environmental effects on seed dormancy dynamics and especially on induction of

secondary seed dormancy, and (ii) dormancy release for buried seeds in a long run. Moreover, in CL OSR hybrids, genetic segregation within F_2 populations as a result of seed impurity or outcrossing pollination might take place, likely resulting in CL OSR volunteers with different zygosities in genes *PM₁* and *PM₂* (see Chapter III of this thesis). Information on the tolerance of these volunteers to herbicides with different modes of action is still limited (Krato et al., 2012). Third, in the context of various field conditions in complex landscapes, a practice-oriented model simulating life cycle of OSR volunteers should be developed for breeders, users and non-users of CL OSR crops to guide stewardship from breeding to sowing, crop management, harvesting, transportation, storage, and volunteer control in the subsequent crops.

Table 1. The most important outcomes and the most important remaining questions that will be focused in the next steps.

New outcomes from the thesis	What we still want to know
1. Dormancy dynamics (primary and potential secondary dormancy) during seed development.	Varietal differences in release of dormancy (especially of secondary dormancy); variety classification based on the speed of dormancy release.
2. Dormancy characteristics of CL OSR seeds; variety classification based on secondary seed dormancy.	
3. Impacts of maternal environment on seed dormancy, e.g. impacts of precipitation during seed development.	Quantification of environmental effects post-harvest on seed dormancy dynamics; loss of primary dormancy and induction of secondary dormancy between harvest and first following tillage (burial experiment with fresh seeds having a high primary dormancy).
4. Impacts of stubble tillage post-harvest with different depths and timings on soil seed bank volunteers of CL OSR (unpublished).	
5. Shallow stubble tillage leads to “survivors” (volunteers in spring which survived stubble tillage and ploughing; unpublished).	Seed fate of harvest lost CL OSR seeds located on the soil surface; e.g. germinating, falling dormancy and death, etc.
6. Impacts of tillage mode and operation time on soil seed bank and volunteers of CL OSR.	Soil seed bank dynamics in a long run in interaction with soil environment and cropping systems.
7. Varietal differences in development of soil seed bank and emergence of volunteers.	
8. Segregation in herbicide tolerance between CL OSR volunteers.	Modelling the life cycle of CL volunteers on the field, including effects of different herbicides on CL volunteers.
9. Integrated strategies to control CL OSR volunteers in following crops; pre- and post-harvest strategies.	

7 Summary

Oilseed rape (OSR) has become the second most important oilseed crop after soybean worldwide, producing 70.95 million tons of seed yield, and providing 13.4% of world supply of oilseeds in 2014. The demand for OSR is expected to increase due to protein meals/cakes used in animal feed and vegetable oils/fats for biodiesel and human consumption. With increasing cultivation area, concern over volunteer OSR is rising, particularly if the variety in question is tolerant to specific herbicides. Currently, the introduction of imidazolinone-tolerant OSR (commercially named Clearfield® OSR; CL OSR) into Europe poses new challenges for chemical control of CL OSR volunteers because of their tolerance to imidazolinone herbicides and other acetolactate synthase (ALS) inhibiting herbicides. Additionally, the potential of gene dispersal in time and space by persistent dormant seeds in the soil and by volunteers is increasing.

Volunteers emerge from the soil seed bank, the volume of which is predominantly dependent on seed dormancy. Therefore, the objectives of this study were (i) to investigate seed dormancy and dormancy formation of CL OSR, and (ii) to find out suitable agricultural strategies to reduce volunteers by growing OSR genotypes with low potential for seed dormancy and seed survival, and by implementing appropriate tillage operations. Focusing on these aims, several experiments were carried out with different methods, namely field experiments, germination tests in the laboratory, and genomic analysis, providing data for three scientific articles.

Experiment 1. A 3-year field trial in south-west Germany investigated dormancy dynamics during seed development (primary dormancy and potential secondary dormancy; tested with an existing standard method in the laboratory) of 10 non-CL OSR varieties (lines) in 2009 and 2010, and of five CL OSR varieties (hybrids) in 2014.

Experiment 2. A total of 15 CL OSR genotypes grown at two locations in south-west Germany in 2012/2013, and eight genotypes (two CL genotypes included) grown at 12 locations across Germany in 2011/2012, were tested for potential secondary seed dormancy with the aim to investigate dormancy traits of CL OSR and maternal environmental effects on dormancy formation.

Experiment 3. A 5-year experiment (2011–2015) was conducted in south-west Germany with non-CL OSR and CL OSR (two CL varieties: high dormant and medium dormant) in the same rotation (non-CL winter oilseed rape - winter wheat - CL winter oilseed rape -

winter wheat - corn) to investigate OSR volunteer dynamics under different modes of tillage (inversion tillage, non-inversion tillage, no-till, with or without additional stubble tillage prior to primary tillage).

Following hypotheses were tested:

> ***Experiment 1.*** *(i) There is primary (innate) and secondary (induced) dormancy in oilseed rape; (ii) primary dormancy decreases during seed development, the potential secondary dormancy increases; (iii) at maturity, the level of the remaining primary dormancy and the varietal potential to secondary dormancy correlate.*

These hypotheses have been approved. Primary dormancy decreased from a high dormancy level (*ca.* 99%) at about 30 days after flowering (DAF) to a quite low level (< 15%) at late seed development. Embryo growth probably regulates the dynamics of primary dormancy, at least during early seed development. Depending on variety and year, potential secondary dormancy initially increased from nearly 0% to the highest level (up to 90%) at about 70 DAF, and then slightly decreased with further seed development. The correlation between primary dormancy and potential secondary dormancy was high at early seed development, but was quite low at late seed ripening.

> ***Experiment 2:*** *(i) There is variation in potential seed dormancy of CL OSR; (ii) F_1 (seeded) and F_2 (harvested) generations of hybrid CL-OSR show similar dormancy levels although changes through environmental effects are known; (iii) the environment (location) during seed development and maturation has an effect on the potential dormancy.*

The hypotheses were approved. The CL OSR genotypes differed in potential secondary dormancy from 0.0 to 95.7% in the F_1 generation and from 3.5 to 77.9% in their corresponding offspring (F_2). Out of the 15 CL genotypes, nine were considered to be low dormant (<30% dormancy level). High correlation ($r = 0.96$) between F_1 and F_2 generations indicates a strong inheritance of seed dormancy. Precipitation during seed development is thought to be a contributor to dormancy formation, e.g. the higher the precipitation the higher the dormancy level. These results indicate that selection or breeding for low dormancy CL OSR is feasible. A direct comparison of varieties by dormancy is only possible if they have been grown and harvested at the same location, due to environmental effects.

> ***Experiment 3:*** *(i) The soil seed bank size of OSR is determined by post-harvest tillage*

(particularly tillage time) and seed dormancy traits of the cultivated variety; (ii) the emergence of volunteers from the seed bank also depends on the mode of tillage; (iii) gene segregation in herbicide-tolerance might occur among CL volunteers.

These hypotheses were partly approved. There was no significant effect of tillage on the soil seed bank, but the soil seed bank was visibly higher if stubble tillage was done prior to primary tillage (179 vs. 56 seeds m^{-2}; treatments with stubble tillage vs. corresponding treatments without stubble tillage). There were significant effects of tillage in general on volunteers in the next crop. Non-inversion tillage resulted in 30 times more volunteers in the following winter wheat crop than inversion tillage due to shallow seed burial depth. A high dormancy OSR variety resulted in a significantly larger soil seed bank than a medium dormancy variety (147 vs. 58 seeds m^{-2}) but in fewer volunteers (0.9 vs. 1.9 volunteers m^{-2}) in the first following crop winter wheat, probably due to slow release of seeds from dormancy. Hypothetically speaking, seeds from low dormancy varieties seem to be released from dormancy more rapidly than seeds from high dormancy varieties. Gene segregation with 10 zygosities of the imidazolinone-tolerance genes *PM1* and *PM2* was detected in the CL volunteers in the first following crop winter wheat. Approximately 90% of sampled plants were homozygous for *PM1* and *PM2*, still conferring a high tolerance to imidazolinones.

Overall, a high variation in potential secondary dormancy was detected for CL OSR, which is similar to non-CL OSR. The contribution of seed dormancy to the soil seed bank was confirmed. During seed development, maternal environment can influence seed dormancy dynamics to some extent. Tillage operations, particularly tillage time, can also influence the soil seed bank and the emergence of volunteers. A very new aspect is that the disposition of seeds to release from dormancy (instead of induction of dormancy) should be considered in further studies. Sound strategies to control volunteers should include (1) the use of low dormancy varieties with a low potential to establish a seed bank and with a fast release from dormancy, and (2) a combination of different tillage operations in the years following OSR cultivation, e.g. delayed inversion tillage with a deep burial depth in the first year, followed by shallow non-inversion tillage in subsequent years.

Combined with a thorough knowledge of seed dormancy, of the development of the soil seed bank and of the release from dormancy, the occurrence of CL volunteers in following crops can be reduced or even avoided by a scope of practical methods and approaches proposed in this study.

8 Zusammenfassung

Raps ist die zweitwichtigste Ölfrucht nach Sojabohnen weltweit. Im Jahr 2014 werden 70,95 Mio Tonnen Raps erzeugt, die 13,4% des globalen Angebots darstellen. Aufgrund der Verwendung von Raps in der menschlichen und tierischen Ernährung sowie für die Herstellung von Biodiesel wird eine Steigerung in der Nachfrage nach Raps erwartet. Mit wachsender Anbaufläche steigt auch die Sorge über möglichen Rapsdurchwuchs, insbesondere wenn die betroffene Sorte Herbizidtoleranz aufweist. Derzeit stellt die Einführung von Imidazolinon-tolerantem Raps (Clearfield®-Raps; CL-Raps) in Europa neue Herausforderungen an die chemische Kontrolle von CL-Durchwuchsraps, da auch andere Herbizide auf Basis von Acetolactatsynthase (ALS)-Inhibitoren nicht mehr oder eingeschränkt wirksam sein können. Außerdem erhöht sich das Potenzial einer zeitlichen und räumlichen Verbreitung der Herbizidtoleranz-Gene mittels überdauernder Rapssamen im Boden und über Durchwuchsraps. Durchwuchsraps geht aus der Bodensamenbank hervor, deren Umfang und Langlebigkeit zu großem Teil von der Keimruhe (Dormanz) abhängt.

Die Ziele der vorliegenden Arbeit waren daher (i) die Keimruhe und die Dormanzausprägung bei CL-Raps zu untersuchen und (ii) geeignete pflanzenbauliche Maßnahmen zur Reduzierung von Durchwuchsraps zu entwickeln. Mit dieser Zielsetzung wurden verschiedene Versuche auf unterschiedlichen methodischen Ebenen durchgeführt, nämlich Feldversuche, Keimtests im Labor und genetische Analysen, die die Grundlagen für drei wissenschaftliche Artikel bilden.

Versuch 1: Ein dreijähriger Feldversuch in Südwest-Deutschland untersuchte die Dormanzdynamik während der Samenentwicklung (primäre Dormanz sowie Disposition zu sekundärer Dormanz; mit Standardmethoden im Labor getestet) von 10 nicht-CL-Rapssorten (Linien) in den Jahren 2009/2010 und von fünf CL-Rapssorten (Hybride) im Jahr 2014.

Versuch 2: Insgesamt 15 verschiedene CL-Rapsgenotypen wurden an zwei Standorten in Südwest-Deutschland im Jahr 2012/13 angebaut, sowie acht Genotypen (einschließlich zwei CL-Genotypen) an 12 Standorten über Deutschland verteilt im Jahr 2011/2012. Diese Genotypen wurden mit einer vorhandenen Standardmethode auf potenzielle sekundäre Dormanz geprüft, mit dem Ziel, die Neigung zur Dormanz von vorliegenden, zum Teil anbaurelevanten CL-Rapssorten zu bestimmen sowie Umwelteffekte während der Samenentwicklung und Abreife auf die Dormanzausprägung zu untersuchen.

Versuch 3: Ein fünfjähriger Feldversuch (2011-2015) wurde in Südwest-Deutschland mit nicht-CL-Raps und CL-Raps (zwei Sorten: hoch dormant und mittel dormant) in derselben Fruchtfolge (nicht-CL-Winterraps – Winterweizen – CL-Winterraps – Winterweizen – Mais) durchgeführt, um die Dynamik von Ausfallraps bei verschiedenen Bodenbearbeitungsverfahren zu untersuchen (wendende und nicht-wendende Bodenbearbeitung – mit oder ohne zusätzlicher Stoppelbearbeitung vor der Grundbodenbearbeitung – sowie „no-till“).

Folgende Hypothesen wurden geprüft:

Versuch 1: *(i) Bei Raps gibt es primäre (innate) und sekundäre (induzierte Dormanz). (ii) Die primäre Dormanz nimmt während der Samenentwicklung ab, die Fähigkeit zur sekundären Dormanz nimmt zu. (iii) Die Höhe der verbliebenen primären Dormanz und das sortenspezifische Potenzial zur sekundären Dormanz korrelieren miteinander zur Samenreife.*

Die Hypothesen ließen sich teilweise bestätigen. Die primäre Dormanz fiel bei allen Genotypen von einem hohen Dormanzniveau (ca. 99 %) etwa 30 Tage nach der Blüte auf ein eher niedriges Niveau (bis zu maximal 15%) während der späten Samenentwicklung zurück. Wahrscheinlich reguliert das Embryowachstum die Dynamik der primären Dormanz, zumindest zu Beginn der Samenentwicklung. Je nach Sorte und Jahr stieg die potenzielle sekundäre Dormanz anfänglich von 0 % etwa 70 Tage nach der Blüte auf das höchste Niveau und sank danach mit fortschreitender Samenentwicklung leicht ab. Die Korrelation zwischen primärer Dormanz und potenzieller sekundärer Dormanz war zu Beginn der Samenentwicklung hoch, aber zur späten Samenreife niedrig.

Versuch 2*: (i) CL-Rapssorten weisen Variabilität im Potenzial für sekundäre Dormanz auf (gering/mittel/hoch dormant). (ii) F_1-(Saatgut) und F_2-(Erntegut) von CL-Raps (Hybridraps) zeigen ähnliches Niveau in der sekundären Dormanz, obwohl Umwelteffekte auftreten können. (iii) Es bestehen Umwelteffekte während der Samenentwicklung und -abreife, die die Dormanzausprägung beeinflussen.*

Diese Hypothesen ließen sich bestätigen. Die CL-Rapsgenotypen wiesen in ihrer potenziellen sekundären Dormanz in der F_1 Generation eine Schwankungsbreite zwischen 0 und 95,7 % und in den dazugehörigen Nachkommen (F_2) zwischen 3,5 und 77,9 % auf. Neun der 15 CL-Genotypen wurden als gering dormant (< 30 % Dormanz) eingestuft. Eine hohe Korrelation ($r = 0,96$) zwischen der F_1 und F_2 Generation deutet auf eine hohe Heritabilität des Merkmals "Dormanz" hin. Die Menge der Niederschläge während der

Samenentwicklung scheint zur Ausprägung späterer Dormanz beizutragen. Eine höhere Niederschlagsmenge in den letzten Wochen vor der Ernte führte zu einem höheren Dormanzpotenzial. Diese Ergebnisse lassen darauf schließen, dass Selektion oder Züchtung auf geringe Dormanz bei CL-Raps möglich ist. Das Dormanzpotenzial von Sorten kann nur direkt miteinander verglichen werden, wenn die Sorten am selben Standort abgereift sind.

Versuch 3. *(i) Der Bodensamenvorrat von Raps wird durch die Bodenbearbeitung nach der Ernte, insbesondere durch den Zeitpunkt der ersten Bearbeitung, und durch die Neigung zu Dormanz der verwendeten Sorte bestimmt; (ii) Der Auflauf von Durchwuchsraps aus der Bodensamenbank ist von der Art der Bearbeitung (wendend/nicht-wendend) bzw. der Bearbeitungstiefe abhängig;(iii) Es kommt zu einer genetischen Aufspaltung der Herbizidtoleranz bei CL-Durchwuchsraps.*

Die Hypothesen ließen sich teilweise bestätigen. Die Bodenbearbeitungsverfahren wendend *vs.* nicht-wendend hatten keinen signifikanten Einfluss auf die Größe der Samenbank, doch die frühzeitige Einarbeitung der Samen mittels flacher Stoppelbearbeitung erhöhte den Bodensamenvorrat sichtbar (179 *vs.* 56 Samen m^{-2} bei den Varianten mit Stoppelbearbeitung *vs.* ohne Stoppelbearbeitung). Die Anzahl an Durchwuchsraps in der Nachfrucht war signifikant. abhängig von der Bodenbearbeitung. Aufgrund der flachen Verschüttung der Samen führte nicht-wendende Bodenbearbeitung zu 30 mal mehr Durchwuchsrapspflanzen in der Nachfrucht Winterweizen als wendende Bodenbearbeitung. Die hoch-dormante Rapssorte führte zu einem signifikant größeren Bodensamenvorrat als die mittel-dormante (147 *vs.* 58 Samen m^{-2}), jedoch zu weniger Durchwuchsraps (0,9 *vs.* 1,9 Pflanzen m^{-2}) in der ersten Nachfrucht. Möglicherweise wird die Dormanz bei Samen von hoch dormanten Sorten im Bodensamenvorrat langsamer gebrochen als bei geringer dormanten Sorten. Die Prüfung der genetischen Aufspaltung für Imidazolinontoleranz (Gene PM_1 und PM_2) im CL-Durchwuchsraps in der ersten Nachfrucht Winterweizen ergab insgesamt 10 unterschiedliche Allelkombinationen. Dabei waren rund 90% der Pflanzen homozygot in PM_1 und PM_2, d.h. sie besaßen eine genetische Disposition für eine hohe Toleranz gegen Imidazolinone.

Insgesamt wurde eine hohe Variationsbreite bei der potenziellen sekundären Dormanz von CL-Raps festgestellt, ähnlich den bereits bekannten Ergebnissen bei nicht-CL-Raps. Der Beitrag der sortenspezifischen Dormanzneigung bei Raps zum Aufbau der Bodensamenbank wurde bestätigt. Während der Samenentwicklung können die

mütterlichen Umweltbedingungen die Dormanzausprägung in einem gewissen Maß beeinflussen. Bodenbearbeitungsverfahren, insbesondere der Zeitpunkt der Bearbeitung, beeinflussen die Bodensamenbank und das Auftreten von Durchwuchsraps in den Folgekulturen. Ein neuer Aspekt ist, dass neben der bisher untersuchten Kontrolle von dormanzinduzierenden Faktoren zukünftig auch Faktoren berücksichtigt werden müssen, die vorhandene Dormanz brechen.

Realistische Strategien zur Kontrolle von Auflaufraps sollten (1) die Verwendung von gering dormanten Sorten mit geringem Potenzial zur Bildung einer Samenbank sowie mit schneller Brechung der Dormanz umfassen, und (2) eine Kombination verschiedener Bodenbearbeitungsverfahren in den Jahren nach dem Rapsanbau beinhalten. Ein Beispiel wäre eine verzögerte, tiefe, wendende Bodenbearbeitung im ersten Jahr mit anschließender flacher, nicht-wendender Bodenbearbeitung in Folgejahren.

Mit dem Hintergrund eines vertieften Verständnisses von Dormanz, der Entwicklung einer Bodensamenbank sowie der Brechung von Dormanz kann das Auftreten von CL-Durchwuchsraps in Nachfrüchten durch die in dieser Arbeit vorgestellten, methodisch breit angelegten und praktischen Ansätze reduziert oder sogar vermieden werden.

9 Reference

Alonso-Blanco, C., Bentsink, L., Hanhart, C. J., Blankestijn-de Vries, H., & Koornneef, M. (2003). Analysis of natural allelic variation at seed dormancy loci of *Arabidopsis thaliana*. Genetics, 164(2), 711-729.

Angelovici, R., Galili, G., Fernie, A.R., & Fait, A. (2010). Seed desiccation: a bridge between maturation and germination. Trends in plant science, 15(4), 211-218.

Auge, G.A., Blair, L.K., Burghardt, L.T., Coughlan, J., Edwards, B., Leverett, L.D. & Donohue, K. (2015). Secondary dormancy dynamics depends on primary dormancy status in *Arabidopsis thaliana*. Seed Science Research, 1-17.

Arc, E., Sechet, J., Corbineau, F., Rajjou, L., & Marion-Poll, A. (2013). ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. Front Plant Sci, 4, 63.

Baskin, C.C., & Baskin, J.M. (1998) Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, California, USA.

Baskin, J.M., & Baskin, C.C. (2004). A classification system for seed dormancy. Seed science research, 14(1), 1-16.

Batlla, D., & Benech-Arnold, R.L. (2004). A predictive model for dormancy loss in *Polygonum aviculare* L. seeds based on changes in population hydrotime parameters. Seed Science Research, 14(03), 277-286.

Becker, H.C., Damgaard, C., & Karlsson, B. (1992). Environmental variation for outcrossing rate in rapeseed (*Brassica napus*). Theoretical and applied genetics, 84(3-4), 303-306.

Beckie, H.J., Séguin-Swartz, G., Nair, H., Warwick, S.I., & Johnson, E. (2004). Multiple herbicide-resistant canola can be controlled by alternative herbicides. Weed Science, 52(1), 152-157.

Belter, A. (2016). Long-Term Monitoring of Field Trial Sites with Genetically Modified Oilseed Rape (*Brassica napus* L.) in Saxony-Anhalt, Germany. Fifteen Years Persistence to Date but No Spatial Dispersion. Genes, 7(1), 3.

Benvenuti, S., Macchia, M., & Miele, S. (2001). Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. Weed Science, 49(4), 528-535.

Bewley J.D. (1997) Seed germination and dormancy. The Plant Cell 9, 1055-1066.

Booth E. J. & Gunstone F.D. (2004) Rapeseed and rapeseed oil: agronomy, production and trade. *In: Rapeseed and Canola Oil: Production, Processing, Properties and Uses (ed FD GUNSTONE), 1st edn, CRC Press, Boca Raton, USA.*

Brimner, T.A., Gallivan, G.J., & Stephenson, G.R. (2005). Influence of herbicide‐resistant canola on the environmental impact of weed management. Pest management science, 61(1), 47-52.

Cardina, J., Herms, C.P., & Doohan, D.J. (2002). Crop rotation and tillage system effects on weed seedbanks. Weed Science, 50(4), 448-460.

Cavalieri, A., Lewis, D.W., & Gulden, R.H. (2014). Pod drop and pod shatter are not closely related in canola. Crop Science, 54(3), 1184-1188.

Çetinbaş, M., & Koyuncu, F. (2006). Improving germination of Prunus avium L. seeds by gibberellic acid, potassium nitrate and thiourea. Horticultural Science, 33, 119-123.

Chono, M., Honda, I., Shinoda, S., Kushiro, T., Kamiya, Y., Nambara, E., Kawakami, N., Kaneko1, S. & Watanabe, Y. (2006). Field studies on the regulation of abscisic acid content and germinability during grain development of barley: molecular and chemical analysis of pre-harvest sprouting. Journal of experimental botany 57, 2421-2434.

Colbach, N., Clermont-Dauphin, C., & Meynard, J.M. (2001). GeneSys: a model of the influence of cropping system on gene escape from herbicide tolerant rapeseed crops to rape volunteers: I. Temporal evolution of a population of rapeseed volunteers in a field. Agriculture, Ecosystems & Environment, 83(3), 235-253.

Reference

Colbach, N., Dürr, C., Gruber, S., & Pekrun, C. (2008). Modelling the seed bank evolution and emergence of oilseed rape volunteers for managing co-existence of GM and non-GM varieties. European Journal of Agronomy, 28(1), 19-32.

Colbach, N., Roger-Estrade, J., Chauvel, B., & Caneill, J. (2000). Modelling vertical and lateral seed bank movements during mouldboard ploughing. European Journal of Agronomy, 13(2), 111-124.

Cuthbert, J.L., & McVetty, P.B.E. (2001). Plot-to-plot, row-to-row and plant-to-plant outcrossing studies in oilseed rape. Canadian Journal of Plant Science, 81(4), 657-664.

Damgaard, C., & Kjellsson, G. (2005). Gene flow of oilseed rape (*Brassica napus*) according to isolation distance and buffer zone. Agriculture, ecosystems & environment, 108(4), 291-301.

Devos, Y., Reheul, D., Schrijver, A.D., Cors, F., & Moens, W. (2004). Management of herbicide-tolerant oilseed rape in Europe: a case study on minimizing vertical gene flow. Environmental biosafety research, 3(03), 135-148.

D'Hertefeldt, T., Jørgensen, R.B., & Pettersson, L.B. (2008). Long-term persistence of GM oilseed rape in the seedbank. Biology Letters, 4(3), 314-317.

Diepenbrock, W., & Geisler, G. (1979). Compositional changes in developing pods and seeds of oilseed rape (*Brassica napus* L.) as affected by pod position on the plant. Canadian Journal of Plant Science, 59(3), 819-830.

Donohue, K. (2009). Completing the cycle: maternal effects as the missing link in plant life histories. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 364(1520), 1059-1074.

Dürr, C., Aubertot, J.N., Richard, G., Dubrulle, P., Duval, Y., & Boiffin, J. (2001). Simple. Soil Science Society of America Journal, 65(2), 414-423.

Fei, H., Ferhatoglu, Y., Tsang, E., Huang, D., & Cutler, A.J. (2009). Metabolic and hormonal processes associated with the induction of secondary dormancy in *Brassica napus* seeds. This paper is one of a selection of papers published in a Special Issue from the National Research Council of Canada-Plant Biotechnology Institute. Botany, 87(6), 585-596.

Fei, H., Tsang, E., & Cutler, A.J. (2007). Gene expression during seed maturation in *Brassica napus* in relation to the induction of secondary dormancy. Genomics, 89(3), 419-428.

Finch‐Savage, W.E., Cadman, C.S., Toorop, P.E., Lynn, J.R., & Hilhorst, H.W. (2007). Seed dormancy release in Arabidopsis Cvi by dry after‐ripening, low temperature, nitrate and light shows common quantitative patterns of gene expression directed by environmentally specific sensing. The Plant Journal, 51(1), 60-78.

Finch‐Savage, W.E., & Leubner‐Metzger, G. (2006). Seed dormancy and the control of germination. New Phytologist, 171(3), 501-523.

Finkelstein, R.R., Tenbarge, K.M., Shumway, J.E. & Crouch, M.L. (1985). Role of ABA in maturation of rapeseed embryos. Plant Physiology 78, 630-636.

Gan, Y., Malhi, S.S., Brandt, S.A., & McDonald, C.L. (2008). Assessment of seed shattering resistance and yield loss in five oilseed crops. Canadian Journal of plant science, 88(1), 267-270.

Garnier, A., Pivard, S. & Lecomte, J. (2008) Measuring and modelling anthropogenic secondary seed dispersal along roadverges for feral oilseed rape. Basic and Applied Ecology, 9, 533-541.

Garello, G., & Le Page-Degivry, M. T. (1999). Evidence for the role of abscisic acid in the genetic and environmental control of dormancy in wheat (*Triticum aestivum* L.). Seed Science Research, 9, 219-226.

Gulden, R.H., Shirtliffe, S.J. and Thomas, A.G. (2003). Secondary seed dormancy prolongs persistence of volunteer canola in western Canada. Weed Science 51, 904-913.

Gulden, R.H., Thomas, A.G. and Shirtliffe, S.J. (2004a). Relative contribution of genotype, seed size and environment to secondary seed dormancy potential in Canadian spring oilseed rape (*Brassica napus*). Weed Research 44, 97-106.

Reference

Gulden, R.H., Chiwocha, S., Abrams, S., McGregor, I., Kermode, A., & Shirtliffe, S. (2004b). Response to abscisic acid application and hormone profiles in spring *Brassica napus* seed in relation to secondary dormancy. Canadian journal of Botany, 82(11), 1618-1624.

Gulden, R.H., Thomas, A.G., & Shirtliffe, S.J. (2004c). Secondary dormancy, temperature, and burial depth regulate seedbank dynamics in canola. Weed science, 52(3), 382-388.

Graeber, K., Nakabayashi, K., Miatton, E., Miatton, E., Leubner-Metzger, G. & Soppe, W.J. (2012). Molecular mechanisms of seed dormancy. Plant, cell & environment, 35(10), 1769-1786.

Gruber, S., Bühler, A., Möhring, J., & Claupein, W. (2010). Sleepers in the soil—vertical distribution by tillage and long-term survival of oilseed rape seeds compared with plastic pellets. European Journal of Agronomy, 33(2), 81-88.

Gruber, S., & Claupein, W. (2006). Effect of soil tillage intensity on seedbank dynamics of oilseed rape compared with plastic pellets as reference material. ZEITSCHRIFT FUR PFLANZENKRANKHEITEN UND PFLANZENSCHUTZ-SONDERHEFT, 20, 273-280.

Gruber, S., & Claupein, W. (2007). Fecundity of volunteer oilseed rape and estimation of potential gene dispersal by a practice-related model. Agriculture, Ecosystems & Environment, 119(3), 401-408.

Gruber, S., & Claupein, W. (2009). Effect of tillage intensity on weed infestation in organic farming. Soil and Tillage Research, 105(1), 104-111.

Gruber, S., Emrich, K., & Claupein, W. (2009). Classification of canola (Brassica napus) winter cultivars by secondary dormancy. Canadian Journal of Plant Science, 89(4), 613-619.

Gruber, S., Feike, T., Weber, E.A., & Claupein, W. (2011). Following the trace of harvesting seed losses-a model to predict the soil seed bank and oilseed rape volunteers. In 13th International Rapeseed Congress, June (pp. 5-9).

Gruber, S., Pekrun, C. and Claupein, W. (2004a). Seed persistence of oilseed rape (*Brassica napus*): variation in transgenic and conventionally bred cultivars. The Journal of Agricultural Science 142, 29-40.

Gruber, S., Lutman, P., Squire, G., Roller, A., Albrecht, H., & Lecomte, J. (2007). Using the SIGMEA data base to provide an overview of the persistence of seeds of oilseed rape in the context of the coexistence of GM and conventional crops. Seed, 4102, 6039.

Gruber, S., Pekrun, C., & Claupein, W. (2004b). Population dynamics of volunteer oilseed rape (*Brassica napus* L.) affected by tillage. European Journal of Agronomy, 20(4), 351-361.

Gruber, S., Pekrun, C., & Claupein, W., 2004c. Reducing oilseed rape (*Brassica napus*) volunteers by selecting varieties with low seed persistence. J. Plant Dis. Prot. (XIX), 151-159.

Gruber, S., Pekrun, C., & Claupein, W. (2005). Life cycle and potential gene flow of volunteer oilseed rape in different tillage systems. Weed Research, 45(2), 83-93.

Gruber, S., Weber, E. A., & Claupein, W. (2014). Which soils are comfortable for oilseed rape seeds (*Brassica napus*) to survive? Plant Soil Environ, 60, 280-284.

Haile, T. A., Gulden, R. H., & Shirtliffe, S. J. (2014). On-farm seed loss does not differ between windrowed and direct-harvested canola. Canadian Journal of Plant Science, 94(4), 785-789.

Haile, T.A. & Shirtliffe, S.J. (2014). Effect of harvest timing on dormancy induction in canola seeds. Weed Science 62, 548-554.

Hattori, J., Brown, D., Mourad, G., Labbé, H., Ouellet, T., Sunohara, G., Rutledge R., King J., & Miki, B. (1995). An acetohydroxy acid synthase mutant reveals a single site involved in multiple herbicide resistance. Molecular and General Genetics MGG, 246(4), 419-425.

Hilhorst, H.W. (1995). A critical update on seed dormancy. I. Primary dormancy. Seed Science Research, 5(02), 61-73.

Hilton, J.R. (1984). The influence of light and potassium nitrate on the dormancy and germination of *avenafatua* l.(wildoat) seed and its ecological significance. New Phytologist, 96(1), 31-34.

Reference

Hossain, S., Kadkol, G.P., Raman, R., Salisbury, P.A., & Raman, H. (2011). Breeding *Brassica napus* for shatter resistance. Abdurakhmonov, 313-332.

Hüsken, A., & Dietz-Pfeilstetter, A. (2007). Pollen-mediated intraspecific gene flow from herbicide resistant oilseed rape (*Brassica napus* L.). Transgenic research, 16(5), 557-569.

Ingram, J. (2000). The separation distances required to ensure cross-pollination is below specified limits in non-seed crops of sugar beet, maize and oilseed rape. Plant Varieties & Seeds, 13(3), 181-199.

Irvine, B., & Lafond, G.P. (2010). Pushing canola instead of windrowing can be a viable alternative. Canadian Journal of Plant Science, 90(2), 145-152.

ISTA 2007. International Rules for Seed Testing. International Seed Testing Association, Iguaçu Falls, Brazil.

Juricic, S., Orlando, S. & Le Page-Degivry, M.T. (1995). Genetic and ontogenic changes in sensitivity to abscisic acid in *Brassica napus* seeds. Plant physiology and biochemistry 33, 593-598.

Kebreab, E., & Murdoch, A.J. (1999). A quantitative model for loss of primary dormancy and induction of secondary dormancy in imbibed seeds of *Orobanche* spp. Journal of Experimental Botany, 50(331), 211-219.

King R.W. 1993. Manipulation of grain dormancy in wheat. Journal of Experimental Botany 44, 1059-1066.

Krato, C., Hartung, K., & Petersen, J. (2012). Response of imidazolinone‐tolerant and‐susceptible volunteer oilseed rape (*Brassica napus* L.) to ALS inhibitors and alternative herbicides. Pest management science, 68(10), 1385-1392.

Krato, C., & Petersen, J. (2012). Gene flow between imidazolinone‐tolerant and‐susceptible winter oilseed rape varieties. Weed Research, 52(2), 187-196.

Laufer, C., Siebachmeyer, M., Gruber, S., Huang, S., Weber, E.A., & Claupein, W. (2014). Against the current-Clearfield® oilseed rape in Germany. Julius-Kühn-Archiv, (443), 720.

Légère, A., Stevenson, F.C., & Benoit, D.L. (2011). The selective memory of weed seedbanks after 18 years of conservation tillage. Weed science, 59(1), 98-106.

Lutman, P.J.W. (1993). The occurrence and persistence of volunteer oilseed rape (*Brassica napus*). Aspects of Applied Biology.

Lutman, P.J.W. (2003) Co-existence of conventional, organic and GM crops – role of temporal and spatial behaviour of seeds. In Boelt B, ed, 1st European Conference on the Co-existence of Genetically Modified Crops with Conventional and Organic Crops, Research Centre Flakkebjerg, pp 33-42.

Lutman, P.J., Berry, K., Payne, R.W., Simpson, E., Sweet, J.B., Champion, G.T., May, M.J., Wightman, P., Walker, K. and Lainsbury, M. (2005). Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (*Brassica napus*). Proceedings of the Royal Society of London B: Biological Sciences 272, 1909-1915.

Lutman, P.J.W., Freeman, S.E. and Pekrun, C. (2003). The long-term persistence of seeds of oilseed rape (*Brassica napus*) in arable fields. The Journal of Agricultural Science 141, 231-240.

Messéan, A., Sausse, C., Gasquez, J., & Darmency, H. (2007). Occurrence of genetically modified oilseed rape seeds in the harvest of subsequent conventional oilseed rape over time. European Journal of Agronomy, 27(1), 115-122.

Mohler, C., J. Frisch & C. McCulloch (2006) Vertical movement of weed seed surrogates by tillage implements and natural processes. Soil and Tillage Research, 86, 110-122.

Momoh, E.J.J., Zhou, W.J. and Kristiansson, B. (2002). Variation in the development of secondary dormancy in oilseed rape genotypes under conditions of stress. Weed Research 42, 446-455.

Morgan, C.L., Ladbrooke, Z.L., Bruce, D.M., Child, R., & Arthur, A.E. (2000). Breeding oilseed rape for pod shattering resistance. The Journal of Agricultural Science, 135(04), 347-359.

Reference

Nambara, E., Okamoto, M., Tatematsu, K., Yano, R., Seo, M., & Kamiya, Y. (2010). Abscisic acid and the control of seed dormancy and germination. Seed Science Research, 20(02), 55-67.

Née, G., Obeng-Hinneh, E., Sarvari, P., Nakabayashi, K., & Soppe, W.J. (2015). Secondary dormancy in *Brassica napus* is correlated with enhanced *BnaDOG1* transcript levels. Seed Science Research, 25(02), 221-229.

Nonogaki, H. (2015). Seed dormancy and germination—emerging mechanisms and new hypotheses. Advances in Seed Biology, 225.

Nunes, A.L., Ascari, J., Pereira, L., Sossmeier, S.G., & Bispo, N.B. (2015). Pod sealant and canola harvest methods for pod shattering mitigation. Australian Journal of Crop Science, 9(9), 865.

Pekrun C., Gruber S., Lutman P., Claupein W. (2003). The potential impact of volunteer rape as a link between previous and current rape crops – its relevance for managing HT-rape. In Boelt B, ed, 1st European Conference on the Co-existence of Genetically Modified Crops with Conventional and Organic Crops, Research Centre Flakkebjerg, pp 187-189

Pekrun, C., Hewitt, J.D.J. and Lutman, P.J.W. (1998). Cultural control of volunteer oilseed rape (*Brassica napus*). The Journal of Agricultural Science 130, 155-163.

Pekrun, C., Lane, P.W., & Lutman, P.J.W. (2005). Modelling seedbank dynamics of volunteer oilseed rape (*Brassica napus*). Agricultural Systems, 84(1), 1-20.

Pekrun, C., Lutman, P.J.W. & Baeumer, K. (1997). Induction of secondary dormancy in rape seeds (*Brassica napus* L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. European Journal of Agronomy 6, 245-255.

Pekrun, C., Lutman, P.J.W., Büchse, A., Albertini, A., & Claupein, W. (2006). Reducing potential gene escape in time by appropriate post-harvest tillage—Evidence from field experiments with oilseed rape at 10 sites in Europe. European journal of agronomy, 25(4), 289-298.

Peltonen-Sainio, P., Pahkala, K., Mikkola, H., & Jauhiainen, L. (2014). Seed loss and volunteer seedling establishment of rapeseed in the northernmost European conditions: potential for weed infestation and GM risks. Agricultural and Food Science, 23(4), 327-339.

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., Gouyon, P.H., & Huet, S. (2008). Where do the feral oilseed rape populations come from? A large - scale study of their possible origin in a farmland area. Journal of Applied Ecology, 45(2), 476-485.

Pivard, S., Demšar, D., Lecomte, J., Debeljak, M., & Džeroski, S. (2008b). Characterizing the presence of oilseed rape feral populations on field margins using machine learning. Ecological Modelling, 212(1), 147-154.

Pfenning, M., Palfay, G., & Guillet, T. (2008). The CLEARFIELD® technology–A new broad-spectrum post-emergence weed control system for European sunflower growers. Journal of Plant Diseases and Protection, Special, (21), 649-653.

Plaza, E. H., Kozak, M., Navarrete, L., & González-Andújar, J. L. (2011). Tillage system did not affect weed diversity in a 23-year experiment in Mediterranean dryland. Agriculture, ecosystems & environment, 140(1), 102-105.

Postma, F. M., & Ågren, J. (2015). Maternal environment affects the genetic basis of seed dormancy in Arabidopsis thaliana. Molecular ecology, 24(4), 785-797.

Price, J.S., Hobson, R.N., Neale, M.A., & Bruce, D.M. (1996). Seed losses in commercial harvesting of oilseed rape. Journal of agricultural engineering research, 65(3), 183-191.

Rajcan, I., & Swanton, C. J. (2001). Understanding maize–weed competition: resource competition, light quality and the whole plant. Field Crops Research, 71(2), 139-150.

Ramezani M. (2007) Environmental fate of imidazolinone herbicides and their enantiomers in soil and water. PhD thesis, University of Adelaide, Adelaide, Australia.

Rathke, G.W., Behrens, T., & Diepenbrock, W. (2006). Integrated nitrogen management strategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (*Brassica napus* L.):

a review. Agriculture, ecosystems & environment, 117(2), 80-108.

Rathke, G.W., Christen, O., & Diepenbrock, W. (2005). Effects of nitrogen source and rate on productivity and quality of winter oilseed rape (*Brassica napus* L.) grown in different crop rotations. Field crops research, 94(2), 103-113.

Rathke, G.W., & Diepenbrock, W. (2006). Energy balance of winter oilseed rape (*Brassica napus* L.) cropping as related to nitrogen supply and preceding crop. European Journal of Agronomy, 24(1), 35-44.

Ray, T.B. (1984). Site of action of chlorsulfuron inhibition of valine and isoleucine biosynthesis in plants. Plant Physiology, 75(3), 827-831.

Romagosa, I., Prada, D., Moralejo, M.A., Sopena, A., Muñoz, P., Casas, A.M., Swanston J.S. and Molina‐Cano, J.L. (2001). Dormancy, ABA content and sensitivity of a barley mutant to ABA application during seed development and after ripening. Journal of Experimental Botany 52, 1499-1506.

Ruisi, P., Frangipane, B., Amato, G., Badagliacca, G., Di Miceli, G., Plaia, A., & Giambalvo, D. (2015). Weed seedbank size and composition in a long‐term tillage and crop sequence experiment. Weed Research, 55(3), 320-328.

Rutledge, R. G., Quellet, T., Hattori, J., & Miki, B. L. (1991). Molecular characterization and genetic origin of the *Brassica napus* acetohydroxyacid synthase multigene family. Molecular and General Genetics MGG, 229(1), 31-40.

Sester, M., Dürr, C., Darmency, H., & Colbach, N. (2006). Evolution of weed beet (*Beta vulgaris* L.) seed bank: quantification of seed survival, dormancy, germination and pre-emergence growth. European Journal of Agronomy, 24(1), 19-25.

Sester, M., Dürr, C., Darmency, H., & Colbach, N. (2007). Modelling the effects of cropping systems on the seed bank dynamics and the emergence of weed beet. Ecological modelling, 204(1), 47-58.

Schatzki, J., Allam, M., Kloeppel, C., Nagel, M., Börner, A. & Möllers, C. (2013a). Genetic variation for secondary seed dormancy and seed longevity in a set of black‐seeded European winter oilseed rape cultivars. Plant Breeding 132, 174-179.

Schatzki, J., Schoo, B., Ecke, W., Herrfurth, C., Feussner, I., Becker, H.C., & Möllers, C. (2013b). Mapping of QTL for seed dormancy in a winter oilseed rape doubled haploid population. Theoretical and applied genetics, 126(9), 2405-2415.

Schlink, S. (1993). Primäre Dormanz bei Körnerrapssorten. Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften 6, 153-156.

Schwabe, S., Gruber, S., Weber, E.A., & Claupein, W. (2016). Vorzüglichkeit des Clearfield®-Systems bei Raps unter verschiedenen Bewirtschaftungsintensitäten. Julius-Kühn-Archiv, (452), 206-209.

Shaner, D.L., Bascomb, N.F., & Smith, W. (1996). Imidazolinone-resistant crops: selection, characterization, and management. Herbicide resistant crops, 143-157.

Shaner, D. L., & O'Connor, S. L. (1991). The imidazolinone herbicides. CRC Press, Inc.

Soltani, E., Soltani, A., Galeshi, S., Ghaderi-Far, F., & Zeinali, E. (2013). Seed bank modelling of volunteer oil seed rape: from seeds fate in the soil to seedling emergence. Planta Daninha, 31(2), 267-279.

Sosnoskie, L.M., Herms, C.P., & Cardina, J. (2006). Weed seedbank community composition in a 35-yr-old tillage and rotation experiment. Weed Science, 54(2), 263-273.

Squire, G.R., Burn, D. & Crawford, J.W. (1997). A model for the impact of herbicide tolerance on the performance of oilseed rape as a volunteer weed. Association of Applied Biologists, 131, 315-338.

Staniland, B.K., McVetty, P.B., Friesen, L.F., Yarrow, S., Freyssinet, G., & Freyssinet, M. (2000). Effectiveness of border areas in confining the spread of transgenic *Brassica napus* pollen. Canadian Journal of Plant Science, 80(3), 521-526.

Reference

Swanson, E.B., Coumans, M.P., Brown, G.L., Patel, J.D., & Beversdorf, W.D. (1988). The characterization of herbicide tolerant plants in *Brassica napus* L. after in vitro selection of microspores and protoplasts. Plant Cell Reports, 7(2), 83-87.

Swanson, E.B., Herrgesell, M.J., Arnoldo, M., Sippell, D.W., & Wong, R.S.C. (1989). Microspore mutagenesis and selection: canola plants with field tolerance to the imidazolinones. Theoretical and Applied Genetics, 78(4), 525-530.

Swanton, C.J., Shrestha, A., Knezevic, S.Z., Roy, R.C., & Ball-Coelho, B.R. (2000). Influence of tillage type on vertical weed seedbank distribution in a sandy soil. Canadian Journal of Plant Science, 80(2), 455-457.

Tan, S., Evans, R.R., Dahmer, M.L., Singh, B.K., & Shaner, D.L. (2005). Imidazolinone - tolerant crops: history, current status and future. Pest management science, 61(3), 246-257.

Thiyam-Hollländer, U., Eskin, N.M., & Matthäus, B. (Eds.). (2012). Canola and Rapeseed: Production, Processing, Food Quality, and Nutrition. CRC Press.

Thomas, D.L., Breve, M.A., & Raymer, P.L. (1991). Influence of timing and method of harvest on rapeseed yield. Journal of production agriculture, 4(2), 266-272.

Totterdell, S., & Roberts, E.H. (1979). Effects of low temperatures on the loss of innate dormancy and the development of induced dormancy in seeds of *Rumex obtusifolius* L. and *Rumex crispus* L. Plant, Cell & Environment, 2(2), 131-137.

U, N. 1935. Genomic analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn. J. Bot. 7:389-452.

Vanasse, A., & Leroux, G.D. (2000). Floristic diversity, size, and vertical distribution of the weed seedbank in ridge and conventional tillage systems. Weed science, 48(4), 454-460.

Weber, E.A., Gruber, S., & Claupein, W. (2014). Emergence and performance of volunteer oilseed rape (*Brassica napus*) in different crops. European Journal of Agronomy, 60, 33-40.

Weber, E.A., Gruber, S., Stockmann, F., & Claupein, W. (2013). Can low-dormancy oilseed rape (*Brassica napus*) genotypes be used to minimize volunteer problems? Field Crops Research, 147, 32-39.

Weber, E.A., Frick, K., Gruber, S., Claupein, W., 2010. Research and development towards a laboratory method for testing the genotypic predisposition of oilseed rape (*Brassica napus* L.) to secondary dormancy. Seed Science and Technology 38, 298-310.

Weiner, J., Griepentrog, H.W., & Kristensen, L. (2001). Suppression of weeds by spring wheat Triticumaestivum increases with crop density and spatial uniformity. Journal of Applied Ecology, 38(4), 784-790.

Wright, K.J., Seavers, G.P., Peters, N.C.B., & Marshall, M.A. (1999). Influence of soil moisture on the competitive ability and seed dormancy of Sinapis arvensis in spring wheat. Weed Research, 39(4), 309-317.

Zhu, Y.M., Li, Y.D., Colbach, N., Ma, K.P., Wei, W., & Mi, X.C. (2012). Seed losses at harvest and seed persistence of oilseed rape (*Brassica napus*) in different cultural conditions in Chinese farming systems. Weed Research, 52(4), 317-326.

Acknowledgements

First of all, I would like to deeply thank **Prof. Dr. Wilhelm Claupein** for offering me the chance to work on the project of Clearfield® oilseed rape and for giving me the valuable supervision. The most profound thanks go to my second supervisor **Prof. Dr. Sabine Gruber,** whose academic, technical, and moral support contributed greatly to this work. Her tireless, kind, patient and vary friendly mentoring made me motivated and focused in the discipline. I deeply appreciate it. Thank you both very much again!

I would also like to deeply thank **Prof. Dr. Jan Petersen** for agreeing to be the second supervisor of this thesis and for the valuable comments and suggestions on my thesis. Also, my thanks go to **PD Dr. Regina Belz** for agreeing to be the third examiner of my oral examination.

I am particularly thankful to **Dr. Ernst Albrecht Weber** for sharing knowledge and guiding me on field trials, and to **Dr. Jens Möhring** for academic support on statistical analysis. In the same context many thanks to **Andrea Rückle** and **Ilona Weikert** at the University of Hohenheim, and to **Sebastian Bökle**, **Andreas Lohrer**, and **Herbert Grözinger** at experimental station Ihinger Hof for their valuable technical support in the laboratory and fields.

Special thanks to my colleagues **Jialu Xu**, **Dr. Sebastian Munz**, **Lisa Schwemmlein**, **Dr. Benjamin Mast**, and **Carolin Weiler** as well as all the staff in our institute for the kind and fantastic support in the past four years.

Last but not least, I want to thank my parents, brothers, sisters-in-law, niece, and nephews for all their warm love, support and motivation throughout the years!

I will always remember these years and wish you all the best in life!

Hohenheim, March 2016

Shoubing Huang

Curriculum Vitae

Personal details

Name	Shoubing Huang
Birth	01 January 1985 in Shandong, China

School Education

1992-1998	Donghuanghaicun Primary School, Shandong, China
1998-2001	Daxiejizhen Junior Middle School, Shandong, China
2001-2005	Juye No.1 Senior High School, Shandong, China

University Education

2005-2009	B.Sc. in Biological Science, Luoyang Normal University, Henan, China
2009-2012	M.Sc. in Agronomy, China Agricultural University, Beijing, China
2012-date	Doctorate candidate in Agronomy (340a), University of Hohenheim, Stuttgart, Germany

Shoubing Huang

Hohenheim
09.03.2016