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

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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

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(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