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Minimal soil disturbance combined with spring cropping can halt soil seedbank accumulation of *Alopecurus myosuroides*

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Abstract

The basic mechanism of soil inversion tillage for control of annual weeds is based on the vertical translocation of weed seeds from the soil surface to deeper soil layers. Buried weed seeds either remain dormant in the soil seedbank and are exposed to biological and chemical decay mechanisms, or they germinate but the seedlings cannot reach the soil surface (fatal germination). However, depending on the seed biology of the respective target species, frequent inversion tillage can lead to a build-up of the soil seedbank. For soil seedbank depletion based on available knowledge of the biology of Alopecurus myosuroides seeds, soil inversion tillage is suggested to be reduced to every third or fourth year with reduced or even no-tillage (direct seeding) in between (rotational inversion tillage systems). Including spring crops in the crop rotation could further help dampening the population growth and hence the seed return into the seedbank. This study investigated the effect of rotational inversion tillage in combination with reduced tillage or direct seeding on the soil seedbank and population development of A. myosuroides. In a long-term field trial, set up in 2012, these tillage strategies were compared with continuous inversion tillage in a 3-year crop rotation with two consecutive years of winter wheat (Triticum aestivum) followed by spring barley (Hordeum vulgare). The results showed a significant decline in the soil seedbank following the spring crop, irrespective of the tillage system. The continuous inversion tillage system and inversion tillage before spring cropping with reduced tillage (shallow tillage with a disc harrow) before winter wheat both led to accumulation of seeds in the soil seedbank. In contrast, inversion tillage before spring cropping with direct seeding of winter wheat depleted the soil seedbank significantly after only one crop rotation. Although only covering one intensively studied field site, these findings highlight the need for diversified cropping systems and indicate potential avenues for reducing soil tillage while controlling economically important weeds.

KEYWORDS

blackgrass, conservation agriculture, direct seeding, rotational tillage, spring barley, winter wheat

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1 | INTRODUCTION

Soil tillage is used to prepare soil for cropping, with the earliest documentation dating back to 3000 BC (Hillel, 1992). Its main effects are soil aeration, loosening of soil compaction and incorporation of crop residues (Skaalsveen et al., 2019; Townsend et al., 2016). Soil tillage is also one of the main pillars of integrated management of *Alopecurus myosuroides* Huds. (blackgrass) and other problematic weeds, in efforts to be less reliant on direct chemical or mechanical weed control measures. Although soil tillage is declared an important pillar of integrated weed management, tillage depth and frequency are rarely adapted to the target weed species biology, but other agronomic parameters like soil aeration and burial of crop residues are the main factors considered for deciding on soil tillage measures.

The basic weed control mechanism with soil tillage is vertical translocation of weed seeds from the soil surface to deeper soil layers. This results in one of two potential fates for buried weed seeds:

- Buried seeds germinate but the seedlings cannot reach the soil surface, a process known as fatal germination (Fenner & Thompson, 2005). Maximum depth of emergence is a function of seed weight, so species with large seeds can tolerate deeper burial than species with small seeds (Bond et al., 1999). Some weed species can germinate from soil depths of 20 cm and more, but most have rather small seeds and are unable to germinate from depths of more than 5 cm below the soil surface (Cousens & Moss, 1990; Froud-Williams et al., 1984);
- 2. Buried seeds remain dormant in the soil seedbank and are exposed to biological and chemical decay mechanisms. The progression of seed decay depends on site-specific pedoclimatic conditions, biological activity in the soil, and seed biology (Gallandt, 2006). The longevity of weed seeds in the soil seedbank is thus speciesdependent and can range from just a few years to several decades (Burnside et al., 1996).

A. myosuroides seeds show a short period of primary seed dormancy, ranging from only a few days to a few weeks after seed maturation (Andersson & Åkerblom Espeby, 2009). The duration of seed dormancy depends mainly on temperature conditions and water availability during the maternal reproductive phase, with warm, dry conditions leading to lower levels of primary dormancy than cool, humid conditions (Menegat et al., 2018; Swain et al., 2006). Light is an important trigger for germination of A. myosuroides seeds (Andersson & Åkerblom Espeby, 2009). The effect of light stimulus on the emergence rate of A. myosuroides is a function of soil aggregate size and burial depth, with the ability to germinate from deeper soil layers being higher in soils with large aggregate size (Cussans et al., 1996). Experiments by Froud-Williams et al. (1984) showed that A. myosuroides germinates mainly at shallow soil depths, with a maximum germination depth of less than 5 cm. The longevity of buried A. myosuroides seeds is 2-5 years, with seed numbers declining to about 3% of the initial level after 3 years (Moss, 1985).

Owing to the mechanisms described above, tillage frequency and tillage depth must be aligned with the seed biology and in particular

seed longevity of the target species, in order to avoid subsequent tillage operations translocating viable seeds to higher soil layers, where they can germinate and emerge.

Beyond the control of weed seeds that are already in the soil seedbank, replenishment of the seedbank needs to be avoided. It is commonly stated that spring cropping is an effective tool for controlling *A. myosuroides* populations and hence for depleting the soil seedbank. However, according to a review by Lutman et al. (2013), there is very little scientific evidence to support this statement. The effects of spring cropping on the population dynamics of *A. myosuroides* have rarely been studied systematically to date, but in the few available studies, plant abundance reductions of up to 98% have been documented (Moss & Hull, 2012; Zeller et al., 2021). The effect of spring cropping on the soil seedbank has not been studied at all so far. However, the indicated significant reductions in plant abundance could successfully prevent the seedbank accumulation during spring cropping years.

It can be concluded that depletion of the soil seedbank could theoretically be achieved by a rotating tillage system (also called strategic tillage system or rotational tillage system) where inversion tillage is limited to every third or fourth year, with reduced or even no-tillage cropping in between (Conyers et al., 2019; Dang et al., 2015a, 2015b). In combination with spring cropping, such rotational tillage systems can be expected to exploit most of the mentioned ecological mechanisms to reduce the soil seedbank.

To fill the raised knowledge gaps, this study investigated the effect of rotational inversion tillage in combination with reduced tillage or no-tillage (direct seeding) on the soil seedbank size and population development of *A. myosuroides* in a 3-year crop rotation comprising two consecutive years of winter wheat followed by spring barley. For this purpose, a long-term field experiment was set up in 2012 and over the course of two full crop rotations, soil seedbank dynamics were compared for the mentioned tillage strategies and a reference continuous inversion tillage system.

2 | MATERIALS AND METHODS

2.1 | Long-term field experiment

The field experiment was established in Scania county in southwestern Sweden (56.2079 N, 12.8619 E), at a site characterised by a temperate oceanic climate (Köppen climate classification Cfb), with a long-term mean temperature of 8.6°C and mean annual cumulative rainfall of 939 mm. Soil texture at the experimental site is classified as loam (22% clay, 30% silt, 48% sand). The site has a long history of heavy infestation with *A. myosuroides* and was specifically chosen due to the homogeneous abundance of this weed. Other weed species occur only in negligible densities. The crop rotation at the site before the start of the experiment consisted of winter wheat, winter oilseed rape, and spring barley.

With the aim to homogenise the soil conditions and weed abundance, the experimental field was ploughed to 25 cm depth in autumn 2011 and spring barley was sown the following spring. After harvest in autumn 2012, a 3-year crop rotation of winter wheat-winter wheat-spring barley was started with the following three different soil tillage systems as experimental factors:

(A) continuous inversion tillage every autumn after crop harvest;

(B) rotational inversion tillage, with shallow tillage before winter wheat and inversion tillage before spring barley;

(C) rotational direct seeding, with direct seeding of winter wheat and inversion tillage before spring barley.

The timing of inversion tillage in System B and C was set to after harvest of the second winter wheat crop in the rotation. By this, buried seeds remain in the soil seedbank for 3 years, making use of the limited seed longevity of the seeds and minimising the potential excavation of viable seeds in following inversion tillage years.

In all systems (A-C), inversion tillage was carried out to 25 cm depth with a standard mouldboard plough. Shallow soil tillage in System B was carried out using a disc harrow to a maximum depth of 10 cm. The same direct seed drill (Väderstad Seed Hawk 30) was used in all plots, for both winter wheat and spring barley and irrespective of soil tillage strategy. All treatments were sown at the same time, at a seed rate of 375 seeds m^{-2} for winter wheat and 350 seeds m^{-2} for spring barley. Winter wheat was sown between early and late September, depending on annual weather conditions, while spring barley was sown in April. The experiment was laid out as a complete randomised block design with four replicate plots (each 12 m \times 24 m) per treatment.

The weed management strategy was the same in all three tillage systems. In autumn, glyphosate (HRAC/WSSA 9; 1104 g ai ha⁻¹ in 150 L water) was used before sowing of winter wheat to control emerged weeds and volunteer crop plants, while prosulfocarb (HRAC/ WSSA 15, 4000 g ai ha⁻¹ in 300 L water) was applied shortly after winter wheat emergence (growth stage BBCH 10-21). In spring, acetolactate synthase inhibitors (HRAC/WSSA 2; comprising a mixture of mesosulfuron [9 g ai ha^{-1} in 200 L water] and iodosulfuron [1.8 g ai ha^{-1} in 200 L water]) were applied (growth stage BBCH 21–32). In spring barley, acetyl-CoA carboxylase inhibitors (HRAC/WSSA 1; fenoxaprop-p-ethyl [69 g ai ha^{-1} in 400 L water]) were used (stage BBCH 13-31). For documenting eventual efficacy failures, herbicide efficacy was monitored 4-8 weeks after application. In addition, the A. myosuroides population was analysed regularly for relevant singlenucleotide polymorphisms that could cause herbicide resistance. The results showed no indications of development of herbicide tolerance or spread of known herbicide resistance alleles in the target population.

2.2 Above-ground crop and weed data collection

The experimental plots were harvested with a standard plot combine harvester. Winter wheat yield was determined in a strip (width 10 m, length 22 m) in the middle of each plot. The straw was left in the plots after harvest.

Plant density of A. myosuroides was determined every year between 2013 and 2020 at the start of A. myosuroides flowering, in WEED RESEARCH & WILFY 117

five randomly positioned 0.25 m² guadrat frames per plot. The five counts per plot were averaged for further analysis.

2.3 Soil seedbank analysis

The seed emergence method was used for estimating the number of viable seeds in the soil seedbank (Mahé et al., 2021). It was selected instead of the seed enumeration method because of the low level of primary and secondary dormancy of A. myosuroides seeds, and hence the low probability of missing ungerminated but viable seeds in the soil samples. Between 2013 and 2018, soil samples were taken to a depth of 25 cm at 30 random positions per plot annually after crop harvest and soil cultivation, using a soil borer with 2.5 cm diameter. These soil samples were separated into depth fractions of 0-5, 5-15, and 15-25 cm. Samples originating from the same plot and soil depth were pooled for further processing. The pooled soil samples were transferred to cultivation trays, to a maximum fill level of 5 cm. The travs were placed in a greenhouse set to 20°C during daytime (16 h) and 12°C at night (8 h) and watered regularly. The soil was stirred once per week, to allow light stimulus to reach the seeds (Andersson & Åkerblom Espeby, 2009). Emerging A. myosuroides plants were counted and removed once per week. When no more plants emerged, the soil samples were stored for 4 weeks at 0°C for cold stratification, and the germination procedure was repeated. The germination trials ended when no more A. myosuroides plants emerged for at least 3 weeks in a row.

2.4 Statistical analysis

Generalised linear mixed models were used for analysing the effect of tillage strategy (A, B, C) on the number of viable seeds per soil depth fraction (0-5, 5-15, 15-25 cm). The experimental year (2012-2018) was used as additional fixed factor in the model, allowing for visualisation of the temporal soil seedbank development. However, since the experimental design is not fully phased (not every crop of the rotation present every year), the factor year is representing differences between crops grown in the respective year but without taking into account differences between years due to environmental variation. Block, representing the variation within blocks (replicates), was used as random factor in the model. For analysis of the effect of the tested soil tillage strategies on the above-ground A. myosuroides population development as well as on crop yield, the same linear mixed model was used, reduced by the factor soil depth. All analyses were performed with R (R Core Team, 2020) and the package Ime4 (Bates et al., 2015).

2.5 Potential caveats of the study

The values presented in the results are means from a 6-year data set, covering two full crop rotation cycles, providing a solid basis for addressing the raised research questions and knowledge gaps. However, it has to be acknowledged that the experiment was not



FIGURE 1 Soil seedbank development between autumn 2012 (before the actual start of the experiment) and autumn 2018 for (A) continuous inversion tillage (mouldboard plough, 25 cm depth), (B) rotational reduced tillage (disc harrow 10 cm depth, inversion tillage before spring barley), and (C) rotational direct seeding (direct seeding of winter wheat, inversion tillage before spring barley). Soil samples were taken in autumn of the respective year, after autumn tillage operations. The harvested crop is indicating the crop harvested before soil seedbank samples were taken. IT, inversion tillage; NT, no-tillage (direct seeding); RT, reduced tillage; SB, spring barley; WW, winter wheat.

performed in different environments, owing to the high costs and effort of maintaining long-term experiments. Furthermore, the experiment layout is not fully phased (presence of each crop of the crop rotation present in each year and treatment), which is why the effect of the respective year cannot be separated from the effect of the treatment factor.

The soil and climate conditions at the experimental site are representative of those in the main regions of abundance of A. *myosuroides* in Europe (Ahmad et al., 2021).

3 | RESULTS

3.1 | Soil seedbank dynamics

Over the course of two full crop rotation cycles (2012–2018) the abundance of viable A. *myosuroides* seeds in the soil seedbank has been studied. The initial topsoil seedbank density in 2012, before the actual start of the experiment, was around 430 viable seeds m^{-2} .

In the continuous inversion tillage system (A), an increase in the number of viable seeds in the topsoil layer (0–5 cm) during the two successive winter wheat cropping seasons was observed (Figure 1, Year 2014 and 2017). In 2013, after the first year of winter wheat, the total number of viable A. *myosuroides* seeds, summed up over all three soil layers, was around 1000 seeds m⁻². After the second year of winter wheat cropping (2014) the total number had increased to well over 2000 seeds m⁻², representing a twofold increase in 1 year. This effect was even more pronounced in the second winter wheat cropping years (2016 and 2017).

A significant decrease in the soil seedbank occurred during spring barley cultivation. After the first season of spring barley cultivation, in autumn 2015, the number of viable seeds per m² in the topsoil layer was reduced by around 70% compared with after the second year of winter wheat cropping in autumn 2014. After the second season of spring barley cultivation (2018), the number of viable seeds in the topsoil seedbank was reduced by around 91% compared with the previous year. The number of viable seeds in the two deeper soil layers (5-15 and 15-25 cm) ranged between <100 and >800 viable seeds m⁻² but not following any obvious pattern.

In System B, reduced tillage before winter wheat and inversion tillage before spring barley, a similar pattern as in System A was observed for the seedbank in the uppermost soil layer (0–5 cm) (Figure 1B). However, the number of viable seeds in the topsoil layer was significantly higher than in system A during the first crop rotation cycle (2013–2015), culminating in over 2000 viable seeds m^{-2} after the second winter wheat season (autumn 2014). Inversion tillage before spring barley caused a significant increase in viable seeds in the 15–25 cm soil layer.

System C, that is, direct seeding of winter wheat and inversion tillage before spring barley, successfully prevented build-up of the seedbank in all three soil layers. A slight increase in viable seeds m^{-2} was observed after every second year of winter wheat cultivation (Figure 1).

A detailed pairwise comparison of the means is given in Table S1.

3.2 | A. myosuroides population development

The observed above-ground A. *myosuroides* population did not follow the same pattern as observed for the soil seedbank. During the first crop rotation, the continuous inversion tillage system (A) showed a stable density of around 50 heads m^{-2} at flowering (Figure 2).



FIGURE 2 Alopecurus myosuroides population changes over time. Values shown are average numbers of A. myosuroides heads m⁻² in summer before crop harvest and from plants that either survived or bypassed weed control measures. SB, spring barley; WW, winter wheat. Bars with different letters are statistically significant different. No letter display is given for tillage strategy C, Years 2017 and 2020, since the measured plant density was 0.

As already indicated by the soil seedbank data, the number of *A. myosuroides* heads m^{-2} in system A declined significantly after the first spring barley cropping season (in 2015).

3.3 | Winter wheat yield

The long-term average yield of winter wheat over the experimental period was about 6 ton ha^{-1} in all treatments. The long-term average yield in System C was around 1.5% lower compared to System A.

4 | DISCUSSION

The results obtained for Systems A and B underline the importance of spring cropping in dampening the build-up of the soil seedbank. Compared to the continuous inversion tillage system, the effect of spring cropping was even more pronounced in System B, where reduced tillage was combined with inversion tillage every third year. The low seed return during spring cropping years was clearly visible by significantly reduced number of seeds in the seedbank the year after, causing a relatively low plant abundance during the following winter wheat cropping season. The underlying mechanism of the observed abundance reduction can probably be found in the germination and emergence pattern of the target species. A. myosuroides germination and emergence occurs in two distinct cohorts, one in autumn and one in early spring (Andersson & Åkerblom Espeby, 2009). Seedbed preparation in spring, before seeding of the spring crop, removes autumn germinated plants effectively, preventing them from producing seeds. Plants that have emerged after crop seeding, can be controlled with selective control methods, further minimising seed production and replenishment of the seedbank. Furthermore it can be assumed that plants of the second cohort produce fewer viable seeds due to the reduced vegetative and generative growth phase compared to autumn germinated plants.

Having conducted a seed burial simulation experiment with plastic beads, Cousens and Moss (1990) concluded that continuous inversion tillage leads to accumulation of seeds at soil depths below 10 cm. This finding could not be reproduced in the present study, where continuous inversion tillage (System A) led instead to accumulation of seeds in the topsoil layer, while the number of viable seeds in deeper soil layers remained more or less unaffected. Potential explanations for this could be that the tested combination of tillage implement and soil conditions did not result in a sufficient deep seed burial.

The rotational reduced tillage System (B) amplified the accumulation of seeds in the topsoil laver. Therefore System B was clearly not able to prevent the A. myosuroides population from growing. Instead, it can be hypothesised that, more or less irrespective of the tillage implement used, sufficient numbers of A. myosuroides seeds accumulated in the topsoil layer, where they have ideal conditions for germination and emergence or where they are protected from biotic and abiotic stressors (Andersson & Åkerblom Espeby, 2009; Cussans et al., 1996). A large soil seedbank in the topsoil layer can rather support several germination and emergence waves, from early autumn until late spring, posing a risk of the population bypassing autumn and/or spring weed control measures. These results are in line with previous findings that shallow tillage, compared with deep tillage, leads to accumulation of seeds in the soil seedbank and hence to higher A. myosuroides plant densities (Dessaint et al., 1997; Zeller et al., 2021). Perhaps, optimisation of the weed control strategy, for example, by adding false seedbed preparation combined with chemical measures, could improve the system.

The results obtained for System C, where direct seeding of winter wheat was combined with inversion tillage before spring barley, deviated significantly from those of Systems A and B. Accumulation of seeds in the topsoil layer was more or less completely avoided, also reflected in the low number of reproductive plants observed at the end of the respective cropping seasons. It can be assumed that weed seeds remaining on the soil surface were exposed to fluctuating

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moisture and temperature conditions and to seed predation, both of which have a negative effect on seed longevity and on the probability of successful germination and plant establishment. A seed predation study, carried out at this experimental site in 2020, observed predation rates of around 89% of available seeds on the soil surface, irrespective of the soil tillage strategy (Daouti et al., 2022). Greater numbers of seeds remaining within the reach of seed predators in System C would partly explain the observed differences compared with Systems A and B. This system outperformed the other two tillage systems in terms of controlling A. myosuroides. Its effectiveness now needs to be validated in large-scale on-farm experiments across different environments.

The observed A. mvosuroides abundance did not reflect the number of viable seeds in the soil seedbank. Thus, as long as weed control measures are reasonably effective, visible weed abundance gives a false picture of the actual situation below ground. The results obtained here, suggest that it may be necessary to include soil seedbank data in any kind of risk assessment and weed management decision support.

The observed long-term winter wheat yield penalty due to direct seeding in System C (1.5%) was well below the observed global average vield loss of 10% (Pittelkow et al., 2015). However, adoption of conservation tillage practices in Scandinavia is still at a very low level (Carter, 2017). Reduced soil tillage in general could help mitigate loss of soil organic carbon (Bohoussou et al., 2022; Krauss et al., 2022) and soil erosion (Montgomery, 2007). It would also lower soil tillage costs and fuel inputs (Uri, 2000). On the other hand, strategic use of inversion tillage reduces the build-up of soil- and stubble-borne diseases and accumulation of nutrients and carbon in upper soil layers (Dang et al., 2015a, 2015b), which are sound agronomic reasons for demand-driven tillage in cereal-dominated production regions (Kirkegaard et al., 2014). Moreover, barriers to adoption of conservation tillage remain high due to required investments in additional machinery (Gould et al., 1989) and potential yield reductions (Lahmar, 2010), as well as the need for acquiring new knowledge (Hydborn et al., 2020). To reduce or avoid yield losses with conservation tillage it is important to follow all three principles of conservation agriculture, that is, to combine no-till with soil coverage with plants or plant residues during the whole year and a diverse crop rotation (Pittelkow et al., 2015).

The results presented here raise future research questions about the effect of rotational tillage at different points in time within the crop rotation and about the interaction of spring cropping and rotational tillage.

Furthermore, the effect of the suggested tillage system on the development of herbicide resistance needs to be investigated. No evidence was found for an increase in target-site resistance (TSR) mutations, nor loss of efficacy of herbicide actives in this long-term experiment. Nevertheless, the gradual evolution of non-target-site resistance (NTSR) within a population can be difficult to detect until numbers of individuals with the trait reach a critical threshold (Somerville et al., 2017). Due to the incomplete resistance characterisation of the studied A. myosuroides population, it cannot be completely excluded that the observed increase in soil seedbank size

is at least partially caused by an increased abundance of individuals bearing NTSR alleles.

Resistance evolution is likely to occur more rapidly where population sizes are larger, providing a greater number of individuals for selection to act upon (Barton, 2010; Délye et al., 2013; Kreiner et al., 2018). Both the continuously ploughed system and the rotational reduced tillage system resulted in large soil seedbanks here, indicative of a large weed population. Given that, it is predicted that populations under these management strategies might evolve resistance more rapidly, despite equal herbicide usage across all three scenarios.

Direct seeding systems are reliant on non-selective herbicide treatments prior to crop seeding. Future changes in legislation and the market availability of such herbicides would complicate the use of direct seeding systems as well it would increase the need for effective post-emergence weed control. A potential compromise could be ultrashallow tillage systems (max. working depth 2-3 cm), allowing for false seedbed preparations before crop seeding which would provide a possibility to replace non-selective chemical methods. However, their effect on soil seedbank dynamics is unclear.

In conclusion, the results suggest that rotational direct seeding prevents build-up of the soil seedbank. Ecological mechanisms such as seed predation and exposure to abiotic stresses significantly reduce the number of viable seeds entering the seedbank. Weed competition effects on the crop can be further reduced when this system is combined with additional integrated weed management tools, such as higher seed rates, competitive crop cultivars and cover crops. Finally, the results demonstrated the efficacy of spring cropping as a tool for control of A. myosuroides, indicating the need for diversification of cereal-based cropping systems. The yield reduction associated with direct seeding could be compensated for by the reduced weed pressure in the system, and hence a reduced need for direct weed control measures and associated labour and energy costs. However, yield reductions due to direct seeding can be expected to be higher in other crops than the ones tested in this experiment, reducing the economic sustainability of rotational direct seeding strategies.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest

DATA AVAILABILITY STATEMENT

All data used for analyses are available from the author upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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