Incorporating agronomic measures into integrated weed management strategies using pre-emergence herbicide cinmethylin to control *Alopecurus myosuroides* Huds.

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1. GENERAL INTRODUCTION

The world population is expected to increase by up to 10 billion people until 2050 (FAO, 2017). In the 21st century, agriculture faces a multitude of challenges: It must produce more food, feed and fibre for a growing population. The global annual demand for the three major cereals, rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), is predicted to be about 3.3 billion tons by 2050 (FAO, 2017). Wheat is the most important source of calories for humans worldwide, representing about 20% of global daily dietary calories, followed by rice (19%) and maize (5%). To avoid hunger and ensure food security, it is necessary to increase yield levels applying a sustainable approach i.e. higher production with rational use of available resources, which also implies responsible use of land and water and enhanced food diversity (Carvalho, 2006).

Crop production can be substantially reduced by abiotic factors such as temperature, water, nutrients and radiation. Additionally, biotic factors including weeds, pathogens and animal pests negatively affect crop production and they account for global potential yield losses of up to 80% (Oerke, 2006). Due to the constant presence of these pests the productivity of crops grown for human consumption is always at risk. Among the pests, weeds are considered as the most problematic biotic constraint to food production. Weeds compete with the crop plants for limited growth resources such as water, light and nutrients and may cause high yield losses of up to 34% (Oerke, 2006). Weeds can also increase production costs if additional cleaning steps are required to remove weed seeds from the harvest products and they may reduce the quality of the final products if they are not removed entirely (Sardana *et al.*, 2017; Oerke, 2006). Moreover, weeds can also serve as a habitat for pests and pathogens (Zimdahl, 2018; Wisler & Norris, 2005; Holzner & Numata, 1982). Crop losses due to weeds can be substantial and may be avoided, or reduced, directly by chemical or mechanical treatments or indirectly by cultural practices. The implementation and combination of these tools is necessary to realize the increased production targets that are required to meet the food demands of the growing world population.

During the last seven decades, herbicides have become the most economical and efficient method for effective weed control strategies. Today, herbicides account for 60% of the pesticides used worldwide (Powles & Shaner, 2001; Dayan, 2019). Weed control was a labour intensive and costly process before the introduction of modern herbicides (Hay, 1974). Through time, weed control technologies have progressed from hand weeding to primitive hoes, animal-powered devices, biological control, and chemical control (Hay, 1974; Kraehmer *et al.*, 2014; Heap, 2014c). Since the discovery of the herbicidal activity of 2,4-dichlorophenoxyacetic acid in the 1940s, herbicides became the main strategy to control weeds in arable crops in developed countries (Wyse, 1992; Heap, 1997; Troyer, 2001; Powles & Shaner, 2001; Dayan, 2019). From the 1950s onwards around 300 active ingredients of herbicides were discovered (Dayan, 2019; Heap, 2014c). Recently, due to the decling acceptability by the society, the authorisation of the herbicides is at high risk, due to contamination of the environment and food chain, health risks to pesticide users and end consumers, a tremendous loss of biodiversity in agro-ecosystems and resistance development (Barth *et al.*, 2007; Kortekamp, 2011; Duke, 2012; Délye *et al.*, 2013; Damalas & Koutroubas, 2016; Gaba *et al.*, 2016). The continuous and repeated use of herbicides with the same mode of action, combined with changes in modern agriculture production (e.g., reduced tillage, monocultures), has led to the selection of herbicide-resistant weeds (Holt, 1992a; Prado *et al.*, 1997; Beckie, 2006; Powles & Yu, 2010). Heap (2014c) defined weed resistance as *"[...] the evolved capacity of a previously herbicide-susceptible weed population to* *survive a herbicide and complete its life cycle when the herbicide is used at its normal rate in an agricultural situation*".

The first herbicide resistant weed was detected in 1970 in the USA for some biotypes of *Senecio vulgaris* L. against PSII-inhibitors (inhibiting the electron transport in photosystem II) (Ryan, 1970). Since then, increasing numbers of herbicide resistant weed species to other modes of action have been documented (Holt, 1992b). In 1978, ACCase (acetyl-CoA carboxylase inhibitors) inhibiting herbicides, which provide selective grass weed control in dicotyledonous crops, launched the market (Kaundun, 2014). The first case of resistance against these herbicides was documented in Australia for *Lolium rigidum* Gaud. biotypes and in England for *Alopecurus mysuroides* Huds. biotypes four years later (Heap & Knight, 1982; Moss & Clarke, 1994). In 1982 a new group of herbicides, known as ALS – inhibitors (inhibiting the activity of the aceto-lactate synthase) was introduced on the market (Saari, 1994). Active ingredients such as chlorsulfuron belonging to the group of ALS inhibitors, were developed for the control of broadleaf weeds in cereals (Saari, 1994). The advantages of chlorsulfuron and other sulfonylurea containing herbicides, increased in popularity among famers because of their efficacy at low application rates. Their high efficacy refers to their specific inhibition of the ALS enzyme and associated low impact on non-target organisms, low residual activity and persistence and high selectivity in different crops (Ray, 1984; Heap, 1997). The first reported case of ALS inhibitor resistance was to chlorsulfuron in *L. rigidum* in Australia (Heap & Knight, 1982). In 1984, the first case of resistance of ALS-inhibitors was reported for *A. myosuroides* in the United Kingdom (Heap, 2021). There are currently 263 known herbicide-resistant weeds that are resistant to 23 out of the 26 existing modes of action (Heap, 2021). Several weed species, including *A. myosuroides*, have evolved multiple resistances to herbicides out of more than one class of active ingredients (e.g. sulfonylureas, aryloxyphenoxy-propionates) belonging to different herbicide groups (ALS-inhibitors, ACCase-inhibitors) with different modes of action (Chauvel *et al.*, 2009; Lutman *et al.*, 2013). The fact that no new mode of action for herbicides has been discovered and approved since the 1990s additionally exacerbates the resistance problem (Duke, 2012).

A. myosuroides is an example of a highly specialized weed species in European cropping systems. This grass weed spread rapidly due to intensive use of herbicides and changes in arable cropping and tillage systems. The increased cultivation of winter annual crops, reduced tillage and early sowing dates of cereals lead to increasing population densities of *A. myosuroides* (Moss, 1990; Melander, 1995; Lutman *et al.*, 2013). The repeated and intensive usage of chemical weed control methods has resulted in herbicide resistance in European countries like England, France, Germany, Belgium, and the Netherlands (Drobny *et al.*, 2006; Délye *et al.*, 2007; Neve, 2007; Heap, 2014c). Herbicide resistance of *A. myosuroides* populations in Europe has been reported mainly against ACCase- and ALS-inhibiting herbicides (Menne & Hogrefe, 2012; Heap, 2014c). As post-emergence herbicide performance has declined, the use of pre-emergence herbicides with active ingredients such as prosulfocarb, flufenacet, pendimethalin, and diflufenican, which tend to be less affected by resistance development, has increased (Menne & Hogrefe, 2012; Dücker *et al.*, 2019). Nevertheless, the development of resistance to these pre-emergence herbicides, which is already increasing with their use, will increase in the future (Menne & Hogrefe, 2012; Bailly *et al.*, 2012).

The industry focuses heavily on research programmes to find novel molecules with new herbicidal modes of action or to develop new uses for existing compounds. Recently, the mode of action of the relatively old herbicide cinmethylin could be clarified (Busi *et al.*, 2020). Cinmethylin targets the fatty acid thioesterases (FAT) in the plastid and has been reported to be effective against *A. myosuroides*, *Apera spica-venti* (L.) Beauv. and *Lolium* spp. in winter cereals (Campe *et al.*, 2018; Busi *et al.*, 2020). However, selection for herbicide resistant populations may occur for this one as rapidly as for other modes of action if it is not combined with other weed control methods. As a component of integrated weed management, the rediscovery of the active ingredient cinmethylin offers great potential, especially with regard to herbicide resistant grass weeds.

Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 aims to achieve sustainable use of pesticides in the EU by reducing the risks and impacts of pesticides on human health and the environment and promoting the use of integrated pest management (IPM). IPM is an ecosystem-based strategy that focuses on long-term prevention of pests or their damage through a combination of comprehensive information on the life cycles of pests and their interaction with the environment and biological, physical and other non-chemical control techniques (Bàrberi *et al.*, 2015; Moss, 2010a). A cornerstone of IPM is integrated weed management (IWM). IWM can be defined as a holistic approach for weed control that combines cultural, biological, physical, and chemical methods of weed control to increase the competitiveness of the crop against weeds (Harker & O'Donovan, 2013; Moss, 2010a). Particularly with regard to the control of *A. myosuroides,* the challenge is to quantify the efficacy and reliability of these different methods in winter cereals. IWM measures have the potential to reduce weed populations to tolerable levels, reduce the negative environmental impact of individual weed control measures, increase the impact of cultural, biological and physical measures and thus at least partially replace herbicides and thereby reduce selection pressure by herbicides on weed populations (Harker & O'Donovan, 2013).

As part of the IWM more attention should be paid to resistance management in the future. An effective resistance management requires the implementation of various measures, including regular monitoring of the fields and investigation of poor control results. Rapid and reliable sensitivity tests offer a possibility to confirm suspected resistance and to allow an appropriate adjustment of the weed control strategy. Most commonly used is the whole-plant pot test in the greenhouse, which provides reliable results, but is time-, space- and labour-intensive (Beckie *et al.*, 2000; Kaundun *et al.*, 2011; Reade & Cobb, 2002). However, several alternative herbicide resistance tests evolved in the last years like the Chlorophyll fluorescence imaging test (Kaiser *et al.*, 2013), Syngenta RISQ test (Kaundun *et al.*, 2011), an enzyme based test which focuses on the increased activity and abundance of glutathione S – transferase (Reade & Cobb, 2002) or the Syngenta Quick Test (Boutsalis, 2001). In contrast to foliage applied herbicides, soil acting herbicides have different requirements for their environmental conditions. Therefore, testing of soil acting herbicides is challenging and needs highly specified methods and a sound interpretation of the results (Menne *et al.*, 2012a).

An important part of IWM measures to control resistant weed populations are cultural methods. Potential cultural practices like stubble cultivation, mechanical weed control, crop rotation, delayed autumn drilling, stubble hygiene, competitive crops, cover cropping, and in-crop cultivations have been listed by Swanton & Weise (1991), Moss & Clarke (1994) and Chauvel *et al.* (2001).

In conventional tillage systems, tillage affects weeds by uprooting, cutting and burying them deep enough in the soil to prevent re-emergence. As a tool of stubble cultivation, ploughing is able to reduce weed populations by vertically displacing most of the freshly shed seeds to a depth of up to 30 cm from which seedlings are unlikely to emerge. In reverse fewer old, buried seed are brought back up to the surface (Moss & Clarke, 1994; Chauvel *et al.*, 2001). In a study of Lutman *et al.* (2013) ploughing was able to reduce *A.*

myosuroides density by an average of 69%. However, a great variability was detected: ploughing could increase populations by 82% or reduce them by up to 95%. The high variability of the control efficacy may be due to the fact that in some cases, ploughing brings more seeds to the surface than it buries, with the consequence that the subsequent weed plant population is higher than where non-inversion tillage has been used (Moss, 2010b). However, re-emerging weed seedlings can be eliminated with an additional soil cultivation (e.g. rotary harrow), which is necessary anyways to allow proper seeding after ploughing. Since many weed species germinate in shallow depths, conservation tillage with shallow soil tillage might result in enhanced weed emergence compared to conventional tillage by ploughing (Wrucke & Arnold, 1985; Colbach *et al.*, 2006; Nichols *et al.*, 2015). Due to the trend towards minimum tillage, *A. myosuroides* populations are facilitated, because *A. myosuroides* germinates from a max. depth of 5 cm (Naylor, 1970). However, shallow tillage can be very effective against annual grass-weeds including *A. myosuroides*. Seeds with no or a short period of primary dormancy are induced to germinate shortly after tillage. Emerged seedlings can then be removed by seedbed preparation for winter cereal (Melander & Rasmussen, 2000; Bond & Grundy, 2001). To ensure success of this strategy, tillage should be delayed until the main germination period has passed. This minimizes the seed bank in the surface layer of the soil and reduces the subsequent emergence of weeds.(Travlos *et al.*, 2020; Bond & Grundy, 2001). As already mentioned by Oliphant J.M. (1977), Rasmussen (2004) and Menegat & Nilsson (2019), false seedbed preparation in combination with late seeding of winter wheat or an herbicide application reduces grassy weeds like *Avena fatua* L. and *Alopecurus myosuroides* effectively in comparison to early seeding with herbicide application. The technique of false seedbed implies the preparation of the soil several days or weeks before drilling or transplanting. Germination of non-dormant weed seeds in the top 5 cm of the soil layer is stimulated. The initial seedbed preparation is then followed by the destruction of emerged weed seedlings by tillage or glyphosate application (Rasmussen, 2004; Merfield, 2015).

Early seeding of winter cereals favors the occurrence of grass weeds like *A. myosuroides* (Moss & Clarke, 1994; Melander, 1995; Lutman *et al.*, 2013; Moss, 2017a). The main germination period of *A. myosuroides* is occurring in early autumn, followed by a minor intensive flush in spring (Moss, 1990). Early seeding of winter cereals in September or early October before *A. myosuroides* has emerged favors its increase. Delayed seeding in combination with the previous seedbed preparation, can already remove the first flush of emerged *A. myosuroides* plants (Menegat & Nilsson, 2019). Delayed sowing of winter cereals can reduce *A. myosuroides* density on average by 31% if soil moisture is sufficient (Lutman *et al.*, 2013). Menegat & Nilsson (2019) achieved a reduction of *A. myosuroides* density of 25% by the combination of false seedbed and delayed sowing.

The implementation of spring crops in the crop rotation creates a crop-free period from fall to spring that favours weed emergence and thus requires additional weed control measures. In temperate cropping systems, cover crops are a potentially effective instrument for IWM in the crop-free fall-to-spring period. Cover crops can replace intensive tillage and herbicides during the fall-to-spring period. Simultaneously, negative impacts of herbicides (contamination of environment and food chain, loss of biodiversity, resistance development) and tillage (soil degradation, soil erosion, reduced biodiversity, nutrient loss) are avoided, while additional benefits (reduced soil erosion by wind and water, recycling and redistribution of nutrients, improve soil structure, increase soil organic matter content, habitat for beneficial insects and microbes) are provided if they emerge quickly after harvest of the previous main crop (Tillman *et al.*, 2004; Ding *et al.*, 2006; Hooker *et al.*, 2008; Harker & O'Donovan, 2013; Blanco-Canqui *et al.*, 2015;

Gerhards & Schappert, 2020, 2020, 2020). Growing cover crops, as well as their mulches, suppress weed growth due to competition for natural resources and allelopathic effects (Farooq *et al.*, 2011; Brust *et al.*, 2014; Kunz *et al.*, 2016; Schappert *et al.*, 2018; Schappert *et al.*, 2019; Pagliai *et al.*, 2004). Cover crop residues also create a physical barrier and can limit weed growth by restricting the light required for weed seed germination (Teasdale, 1996; Fisk *et al.*, 2001). The success of cover crops as an IWM practice, is related to a fast emergence and high soil cover, which depends on the chosen species, soil properties, and the weather conditions at the field location (Blanco-Canqui *et al.*, 2015; Constantin *et al.*, 2015). Under favourable growing conditions, cover crops can provide weed control efficacies of up to 95% even for grass weeds such as *A. myosuroides* (Schappert *et al.*, 2018).

However, the efficacy of the selected cultural measures may vary with the degree of infestation, previous cropping history, type and efficiency of tillage, implements used, which affects the distribution depth of weed seeds in the soil, soil structure and moisture, and weather conditions both before and after tillage. The aim of IWM should be to maximize utilization of non-chemical weed control through suitable cultural practice and other preventive methods This in turn allows the use of herbicides as a targeting (last) measure and delays/prevents the development of herbicide resistance. Therefore, further research needs to be conducted to find the most successful control strategy for *A. myosuroides.*

1.1. Objectives of the Thesis

The holistic approach for integrated management of *A. myosuroides* includes monitoring, as well as preventive, biological, and chemical methods. The efficacy of each method was investigated within this this. The objectives of this thesis were

1. to develop a new and quick sensitivity test system for two pre-emergence herbicides (flufenacet, cinmethylin) allowing the detection of putative resistance
2. to estimate the ability of selected biological, mechanical, and chemical weed control practices on weed suppression before spring cropping
3. to test the efficacy against *A. myosuroides* and crop response of the new pre-emergence herbicide cinmethylin in winter wheat compared to other pre- and post-emergence herbicides
4. to test the efficacy against *A. myosuroides* and crop response of the new soil residual herbicide cinmethylin in winter wheat combined with different stubble treatments
5. to test the efficacy against *A. myosuroides* and crop response of the new soil residual herbicide cinmethylin in winter wheat combined with different stubble treatments and seeding dates

1.2. Structure of the Thesis

The current thesis consists of four chapters proposing several approaches of integrated weed management in winter cereals. The first chapter represents the General Introduction, in which the structure of the thesis is presented and the objectives pursued in this thesis are emphasized. The second chapter comprises **five scientific papers,** which were published or submitted in peer-reviewed journals.

The first section of the second chapter presents the article "**Development of an agar bioassay sensitivity test in *Alopecurus myosuroides* for the pre-emergence herbicides cinmethylin and flufenacet"** and was published to the journal *Agronomy,* it describes the development and evaluation of a new agar-based sensitivity test system for detecting herbicide resistance to pre-emergence herbicides in *A. myosuroides* populations.

The second article is titled **"Weed Suppressive Ability of Cover Crop Mixtures Compared to Repeated Stubble Tillage and Glyphosate Treatments"** and was published in the journal *Agriculture*. It presents the weed control efficacy of cover crop mixtures, when they are sown using either as a mulch or no-till system, compared to various mechanical stubble tillage treatments and a glyphosate application during the crop-free period in autumn and winter.

The third article is titled "**Effect of cinmethylin against *Alopecurus myosuroides* Huds. in winter cereals"** and was published in *Plant, Soil and Environment*, and describes two field experiments over a period of three years dealing with pre-emergence herbicide application and delayed winter cereal seeding. The effects of these two parameters on *A. myosuroides* control efficacy and cereal grain yield were analysed.

The fourth article is titled "**Exploring the effects of different stubble tillage practices and glyphosate application combined with the new soil residual herbicide cinmethylin against *Alopecurus myosuroides* Huds. in winter wheat**" and was submitted in the journal *Agronomy*. It presents the effect of different stubble and glyphosate treatments and pre-emergence herbicide application on *A. myosuroides* and winter wheat density as well as the impact on crop yield.

The fifth article is titled **"Effects of stubble treatments, delayed sowing and pre-emergence cinmethylin application on *Alopecurus myosuroides* Huds. density and cereal grain yield"** and was submitted in the journal *Weed Research*. It presents the effect of cinmethylin in combination with stubble treatments and delayed drilling of winter annual cereals on *A. myosuroides* and the impact on crop yield.

The findings of each paper are discussed in the section General Discussion (chapter 3). A comprehensive Summary (chapter 4) of the entire thesis is provided after the General Discussion. Apart from the peer-reviewed journal articles included in the thesis, one additional contribution was given as a poster presentation at an international scientific conference during the course of this work. This work was supplementary to the included articles, and therefore not included in the current thesis.

Messelhäuser M. H., Schappert A., Saile M., Peteinatos G. G., Gerhards R. (2019) Black-grass control efficacy and yield response in spring barley after cover cropping, repeated stubble tillage and glyphosate treatments. In: *20th Conference of the Hellenic Weed Science Society*. Weed Research: Problems, trends and current challenges. 4-6 April 2019. Hellenic Weed Science Society. Agrinio, Greece.

2. PUBLICATIONS

2.1. Development of an agar bioassay sensitivity test in *Alopecurus myosuroides* for the pre-emergence herbicides cinmethylin and flufenacet

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2.1.1. ABSTRACT

Rapid and reliable tests for pre-emergence herbicide susceptibility in weeds are important to test a wider range of accessions on their baseline sensitivity, as well as to provide information on putative resistance. This study focused on the development of an agar quick test to determine sensitivity differences in *Alopecurus myosuroides* Huds. to pre-emergence herbicides containing flufenacet and cinmethylin. The new agar quick test and a standardized whole plant pot bioassay were conducted twice in 2019. For both test systems, seeds of 18 populations of *A. myosuroides* originated from Southwest Germany and Great Britain were used and treated with discriminating rates of herbicides in dose-response studies. After 28 days, the above-ground dry matter of the plants was determined and the resistance factors were calculated. The agar test was able to provide information on the resistance status of the tested biotype within 12 days. All populations did not show reduced sensitivity to cinmethylin. Within three populations, differences in sensitivity levels were observed between the two test systems. As cinmethylin is not yet marketed in Europe, these resistance factors can also be considered as a baseline sensitivity for *A. myosuroides*. For flufenacet, the resistance factors differed significantly from the whole plant pot bioassay and the agar test for the biotypes A (0.35, 13.1), C (0.56, 13.2), D (1.87, 12.4), E (15.5, 3.5) and H (2.95, 14). It was possible for the most part for the cinmethylin tested populations to confirm the results of the standardized whole plant pot bioassay in the agar bioassay sensitivity tests, and hence create a promising, faster test system.

KEYWORDS

Whole plant biotest, quick test, Black-grass, herbicide resistance detection, VLCFA, new mode of action

2.1.2. INTRODUCTION

Herbicides are most effective in controlling weeds in modern agricultural systems and hence in safeguarding and maintaining crop yield and quality. Due to the shortage of new modes of action since the 1990ies, the range of herbicides for weed control is even more limited (Rüegg *et al.*, 2007; Chauvel *et al.*, 2009; Lutman *et al.*, 2013; Dayan, 2019)

The repeated use of herbicides with the same mode of action create resistant individuals within the population and significantly reduces weed control efficacy (Gressel & Segel, 1990; Hull & Moss, 2012). Currently there are 262 known herbicide-resistant weeds that are resistant to 23 of the 26 existing modes of action (Heap, 2020). Several weed species including *Alopecurus myosuroides* Huds. (Blackgrass) express multiple resistance to herbicides with different modes of action (Chauvel *et al.*, 2009; Lutman *et al.*, 2013). Many *A. myosuroides* populations in Europe are resistant to post-emergent herbicides inhibiting the acetyl CoA carboxylase (ACCase) and acetolactate synthase (ALS) (Menne & Hogrefe, 2012; Heap, 2014b). Therefore, *A. myosuroides* is considered one of the most troublesome weed species in Europe. Effective control of *A. myosuroides* is necessary to maintain the productivity of arable farming.

Pre-emergence herbicides (e.g. Prosulfocarb (HRAC N/15), flufenacet (HRAC K3/15) or pendimethalin (HRAC K1/3)) are less affected by resistance (Bailly *et al.*, 2012). However, their activity depends on sufficient soil moisture content to guarantee herbicide uptake via the roots. Flufenacet showed high efficacy against *A. myosuroides* populations that were resistant to PSII-, ACCase- and ALS-inhibitors. It inhibits the synthesis of the very long chained fatty acids (VLCFA) in the endoplasmic reticulum by disrupting the elongation process of the fatty acids (Busi, 2014; Dücker *et al.*, 2019). Previous studies have confirmed resistance to flufenacet in several populations of *A. myosuroides* (Menne *et al.*, 2012a; Dücker *et al.*, 2019).

The pre-emergent herbicide cinmethylin, is a promising new herbicide for the control of *A. myosuroides* in cereals. Originally cinmethylin was developed by the Shell Chemical Company as a herbicide against grass weeds in rice (Dayan, 2019). Since 2018, the mode of action of cinmethylin could be clarified, it inhibits the fatty acid thioesterases (FAT) in the plastid (Campe *et al.*, 2018). Thus, the transfer of fatty acid from the plastid to the endoplasmic reticulum is disturbed in sensitive plants (Campe *et al.*, 2018). Similar to other pre-emergent herbicides, it is absorbed predominantly by the roots of the plants. It is most effective against problematic grass weeds such as *A. myosuroides*, *Apera spica-venti* and *Lolium spp*. in winter cereals (Basf, 2018). Since cinmethylin has not been used in Europe and acts with a new mode of action, it is assumed that *A. myosuroides* are still susceptible to this herbicide.

A sustainable resistance management requires the implementation of various measures, including a regular monitoring of the fields and investigations on the reason of any poor control. Quick and reliable sensitivity tests are necessary, to confirm putative resistance and allow for an adequate adaptation of the weed control strategy. Pre-emergence herbicides have certain requirements for their environment. Therefore, testing of soil acting herbicides is sophisticated and needs specific methods and a considered interpretation of the results (Menne *et al.*, 2012a).

The most common herbicide resistance test is the whole plant pot bioassay in the greenhouse (Beckie *et al.*, 2000). Mature seeds of plants that survived regular herbicide applications are collected in the field. After primary dormancy has been broken, seeds, as well as a sensitive biotype, are sown in pots filled with soil and treated with different dosages of herbicides. After 14 and 28 days, visual assessments of herbicide efficacy are carried out. Optionally, the fresh or dry matter is also recorded (Beckie *et al.*, 2000; Menne & Hogrefe, 2012). The whole plant pot bioassay is time- and space-consuming and labour-intensive (Reade & Cobb, 2002; Kaundun *et al.*, 2011). Another seed based method for the detection of resistance is a petri dish assay (Murray *et al.*, 1996; Moss, 1999). A less widespread test is a pollen bioassay (Letouzé & Gasquez, 2000). But there is also a bunch of tests which generate results more quickly. The enzyme based test of Reade & Cobb (2002) which focuses on the increased activity and abundance of glutathione-S–transferase or the Syngenta Quick – Test (Boutsalis, 2001). An agar based method for post – emergence herbicides, called Syngenta 'RISQ' test was published by (2011). This test is described as a 'Resistance In – Season Quick' test, where grass seedlings are grown on herbicide containing agar. Visual assessment is done 10 days after transplanting. Furthermore the results of the whole plant pot bioassay depend on many factors such as soil properties (pH, organic matter and clay content), temperature regimes and irrigation which determine the bioavailability of herbicides (Beckie *et al.*, 2000; Menne *et al.*, 2012a) which not always delivers consistent results.

Also a baseline sensitivity data can be created prior to the initial introduction of a herbicide active ingredient, which helps to evaluate changes in sensitivity to the herbicide and thus the early detection of resistance developments (Ulber *et al.*, 2013). However, cross-resistance may also exist for new herbicides due to existing resistance mechanism affecting also new molecules (Ulber *et al.*, 2013; Espeby *et al.*, 2011). The baseline sensitivity describes the variation of the sensitivity of different accessions and could provide information, whether the new active substance cinmethylin is affected by existing resistance mechanisms.

Therefore, the objective was to develop a new and quick sensitivity test system for two pre-emergence herbicides (flufenacet, cinmethylin) allowing the detection of putative resistance. We hypothesize that (i) the agar bioassay sensitivity test is faster than the whole plant pot bioassay, (ii) the new agar bioassay sensitivity test will deliver similar herbicide resistance factors (RFs) than the whole plant pot bioassay, (iii) the results of both test systems were more consistent for cinmethylin than for flufenacet.

2.1.3. MATERIALS AND METHODS

Four experiments were carried out in spring 2019: two whole plant pot bioassays in the greenhouse and two agar tests in the laboratory inside a climate chamber under controlled environmental conditions.

2.1.3.1. Origin of the seed samples

Seed samples of 18 *A. myosuroides* populations (Sens, A–Q) were tested and compared with each other. Seed samples of 11 *A. myosuroides* populations (A–L) were collected from 11 fields located in Southwest Germany in the region of Stuttgart (48°47′ N, 9°11′ O), Karlsruhe (49°0′ N, 8°24′ O) and Tübingen (48°31′ N, 9°3′ O) whereby flufenacet products were applied in at least three cropping seasons before seed harvest, according to field history data. The populations A–J were suspicious of increased tolerance against flufenacet. The seed samples of the populations K and L are from fields without any herbicide resistance problems.

The populations O–Q originated from BASF Germany, and showed reduced sensitivity to flufenacet. These seed samples were collected from fields in Southwest Germany in the region of the Limburgerhof (49°25′ N, 8°24′ O).

The selected populations were compared with samples of three commercially available populations, including a multi-resistant population of M, as well as the resistant population of N and the population Sens, which is commonly used as a sensitive population for resistance testing with ALS-, ACCase- and VLCFA inhibitors. These seeds were purchased from Herbiseed Ltd., Berkshire, UK Seeds. All seed samples from fields were collected by the end of June in 2017 and 2018. Therefore, within each field, 30 seeds were randomly chosen *A. myosuroides* plants were collected and stored in a paper bag at 7 °C.

2.1.3.2. Determination of discriminating rates of herbicides

For the conducted whole plant pot bioassay in the greenhouse, the discriminating herbicide doses were determined based on the max. field dose rates of cinmethylin (500 g a.i. ha^{-1}) and flufenacet (250 g a.i. ha^{-1}). Dose-response tests were conducted for the agar bioassay sensitivity test to define the herbicide concentrations that best correlated with the discrimination rates of cinmethylin and flufenacet in the whole plant pot tests. First, the growth of the standard-sensitive *A. myosuroides* population Sens was tested at a wide range of herbicide concentrations (2 nmol a.i. µL–0.002 nmol a.i. μL^{-1}). After this primary screening, a tighter range of herbicide concentrations were tested with populations of L and N. These tests were repeated, and the herbicide concentrations were further reduced until discriminating herbicide concentrations were found. A discriminating concentrations is the concentration of herbicide that gives the greatest vertical discrepancy between the dose-response curves of the R and S biotypes (Beckie et al., 2000). Finally, at a concentration of 0.24 pmol a.i. uL^{-1}, all *A. myosuroides* plants should be controlled by the appropriate herbicide.

2.1.3.3. Whole plant pot bioassay in greenhouse

Dose response studies with seeds of all 18 *A. myosuroides* populations were implemented in a greenhouse whole plant pot bioassay. To achieve a uniform seedling density, germination tests were performed prior to the greenhouse trial. According to the germination test, 10–84 seeds were sown to gain a density of 10 plants per pot. All of the seed samples were sown in compostable pots with an edge length of 8 cm (512 cm³), filled up with a soil substrate containing 60% silt, 11.3% sand, and 28.7% clay with a C content of 2.3%. Seeding depth was 2 cm. Herbicides were applied five days after seeding. All populations were treated with flufenacet (Cadou® SC, 508.8 g a.i. L^{-1}, Bayer AG) and cinmethylin (750 g a.i. L^{-1}, BASF SE). Plants were treated with nine different dosages (0.00; 3.91; 7.81; 15.63; 31.25; 62.5; 125; 250; 500; 1000 g a.i. L^{-1} flufenacet) (0.00; 3.87; 7.73; 15.47; 30.94; 61.88; 123.75; 247.5; 495; 990 g a.i. ha^{-1} cinmethylin) including a control, treated with water only. Herbicide treatment was carried out with a precision application chamber using a single flat fan nozzle (8002 EVS, TeeJet Spraying Systems Co., Wheaton, IL, USA, pressure 300 kPa, water amount 200 l ha^{-1}, speed 700 mm s^{-1}). The pots were arranged in a randomized complete block design with three repetitions per treatment. In total, 30 *A. myosuroides* plants per herbicide and dosage were treated. All of the *A. myosuroides* plants were cultivated in the greenhouse for 33 days with a 16 h photoperiod at 18 °C and an 8 h dark period at 10 °C and a humidity of 55%. For illumination, sodium vapor lamps (400 W) were used. Visual assessment of herbicide efficacy damage was carried out 14 days and 28 days after treatment, compared to the untreated control. After 28 days above ground, biomass was harvested at soil level and dried at 80 °C for 72 h. Afterwards dry matter content has been determined.

2.1.3.4. Agar bioassay sensitivity test

Eighteen different *A. myosuroides* populations were tested for sensitivity to the active ingredients of flufenacet and cinmethylin in an agar bioassay sensitivity test. Experimental design was a randomized complete block design with four repetitions per treatment. To get 40 *A. myosuroides* plants per herbicide and dosage, 10 germinated seeds of each biotype were transferred to one plastic tray. To ensure homogenous growth stage and reduce variation due to dormancy and numb seeds, pre-germinated seedlings were transplanted. Therefore, *A. myosuroides* seeds were pre-germinated on a moist filter paper in a climate chamber with a 16 h photoperiod at 18 °C and an 8 h dark period at 10 °C. After 5 days, seeds had a coleoptile of around 2 mm (BBCH 05–09) (Figure 2.1.3-1 A). For the agar bioassay sensitivity test, plastic trays with an edge length of 11 × 11 cm and a height of 6 cm were prepared with 200 g of fire dried quartz sand (0.7–1.2 mm). The germinated seeds were transferred and placed with the help of a tweezer on the dry sand in the plastic trays (Figure 2.1.3-1 B).

(A) (B)

*Figure 2.1.3-1: State of development of the A. myosuroides seed at the time of transplantation (**A**). Seeds were placed with the shot upwards on the sand (**B**).*

All populations were tested over a range of seven concentrations for flufenacet 0; 0.004; 0.008; 0.01; 0.03; 0.06; 0.24 pmol a.i. ul^{-1} and for cinmethylin 0; 0.008; 0.02; 0.03; 0.06; 0.12; 0.24 pmol a.i. ul^{-1}. The control was treated with water only. For herbicide application, agar-herbicide pads were prepared right before usage. One agar pad included 25 mL of distilled water mixed with the related amount of herbicide. Furthermore 25 mL of 60 °C warm agar solution (3.2% agar) (European type, CHEMSOLUTE, Renningen, Germany) and 0.05% of a nutrient solution (8% nitrogen, 8% phosphate and 6% potassium) were added and mixed homogenously. The final mixture was poured into a form with a size of 11 × 11 cm to generate equal sized pads with a height of 1 cm (Figure 2.1.3-2). After cooling down, the pads were inserted into the already prepared plastic trays with the seeds. The pads were placed with direct contact to the germinated plants (Figure 2.1.3-3). At the end, every plastic tray was covered with a transparent lid All trays were placed for 7 days in a climate chamber at a temperature of 20 °C, with 12 h of illumination (24 W m^{-2}) (Figure 2.1.3-4). After 7 days above ground, biomass was harvested at soil level and dried at 80 °C for 72 h. Afterwards, dry matter content has been determined.

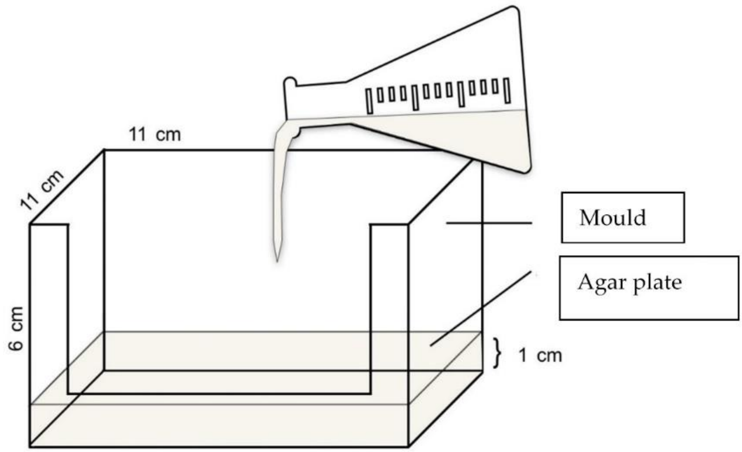

Figure 2.1.3-2: A schematic drawing of the mold for agar herbicide plates.

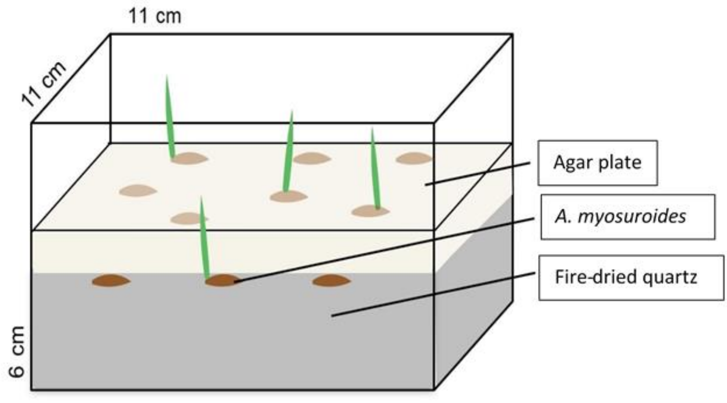

Figure 2.1.3-3: Structure of the agar test: 1st layer = agar plate consisting of agar, herbicide and nutrients; 2nd layer = pre-germinated A. myosuroides seeds; 3th layer = fire-dried quartz sand.

Figure 2.1.3-4: Structure of the agar test: 1st layer = fire-dried quartz sand; 2nd layer = pre-germinated A. myosuroides seeds; 3rd. layer = agar plate consisting of agar, herbicide and nutrients. Plants showed in the picture are from the sensitive standard treated with no herbicide, 7 days after transplanting.

2.1.3.5. Statistical analysis

Dose-response assays to characterize the resistance levels of flufenacet and cinmethylin were analyzed as randomized block designs. The data were analyzed with the statistical software R Studio (Version 3.6.2, RStudio Team, Boston, MA, USA). To illustrate differences of herbicide sensitivity between populations, the R package drc was used to calculate dose response curves (DRC) and ED50 values for each *A. myosuroides* population and herbicide treatment based on relative dry matter (Version 3.0-1) [25]. To determine the response of 18 *A. myosuroides* populations to different dose rates of flufenacet and cinmethylin, only populations without significant differences (based on 95% confidence intervals) between the two experiments conducted in the greenhouse and the climate chamber were included in the analysis. DRCs were calculated with a three parametric log-logistic model (1) according to (Streibig, 1988).

$$\mathrm{Y} = \mathrm{c} + \frac{D - C}{1 + exp\left(b\ ln\left(\frac{x}{ED50}\right)\right)} \qquad (1)$$

where Y represents the plant response (relative dry matter content (g)), D is the upper limit of the curve, C is the lower Limit and b is proportional to the slope around ED50, the dose at which the plant response is reduced by 50%. Shifts of the dose-response curves were horizontally assessed by F-test, (α = 0.05). In all evaluations, a model lack of fit test was performed (Knezevic *et al.*, 2007). Finally, the herbicide resistance factor (RF) was calculated:

$$\mathrm{RF} = \frac{ED50_{test\ biotype}}{ED50_{standard}} \qquad (2)$$

where ED50, as mentioned above, is the herbicide dosage causing 50% reduction in the plant response (Knezevic *et al.*, 2007).

2.1.4. RESULTS

2.1.4.1. ED50 values and resistance factors - cinmethylin

In the whole plant bioassay, the susceptible *A. myosuroides* standard (Sens) showed for cinmethylin a 50% definite reduction in relative dry matter at 10.05 g a.i. ha^{-1} in the first experiment (V1) and at the second (V2) 15.32 g a.i. ha^{-1}. For population F the ED50 V2 value was increased by 211% (265.04 g a.i ha^{-1}) compared with V1. The calculated resistance factor (RF) for population F in V1 was 8.5 and for V2, 17.3. Population J showed an increased ED50 value in V2 by 67% (250.48 g a.i ha^{-1}) compared to V1. Therefore, an RF of 14.95 was calculated in V1 and 16.35 for V2. The 50% reduction in relative dry matter content in all of the other populations ranged between 1.5 g a.i ha^{-1} until 48.68 g a.i. ha^{-1} in V1 and 0.5 g a.i ha^{-1} until 59.29 g a.i ha^{-1} in V2. V1 and V2 in the greenhouse did not differ significantly from each other (Table 2.1.4-1).

Table 2.1.4-1: Evaluation of sensitivity of the A. myosuroides populations to cinmethylin in a whole plant pot bioassay in greenhouse and agar based resistance test under controlled environmental conditions in a climate chamber. ED50 = herbicide dosage causing 50% reduction in the relative dry matter content and RF = resistance factor. V1 = experiment one, V2 = experiment 2. Whole plat pot bioassay V1: lack-of-fit, p *= 0.03; V2: lack-of-fit, P = 0.02; Agar bioassay sensitivity test V1: lack-of-fit, P = 0.005; V2: lack-of-fit, P = 0.003.*

Cinmethylin								
	Whole Plant Pot Bioassay				**Agar Bioassay Sensitivity Test**			
Population	**ED50 V1**	**ED50 V2**	**RF V1**	**RF V2**	**ED50 V1**	**ED50 V2**	**RF V1**	**RF V2**
	g a.i. ha^{-1}				**pmol a.i. L^{-1}**			
Sens	10.05	15.32	1.00	1.00	0.22	0.20	1.00	1.00
A	13.57	19.15	1.35	1.25	0.10	0.17	0.44	0.78
B	14.48	30.64	1.45	2.00	0.30	0.34	1.34	1.56
C	19.20	30.33	1.91	1.98	0.10	0.09	0.46	0.40
D	11.48	17.31	1.14	1,13	0.19	0.18	0.84	0.84
E	35.55	59.29	3.54	3.87	1.35	0.35	2.20	1.60
F	85.20	265.04	8.50	17.30	1.37	0.55	7.12	2.50
G	12.08	26.04	1.20	1.70	0.88	0.24	0.33	1.10
H	48.68	53.62	4.85	3.50	1.11	0.33	3.75	1.50
I	17.33	28.65	1.73	1.87	1.01	0.22	0.72	1.00
J	150.00	250.48	14.95	16.35	0.79	0.28	14.16	1.26
K	20.70	29.57	2.06	1.93	1.25	0.28	0.81	1.25
L	35.58	59.29	3.54	3.87	0.30	0.32	1.35	1.6
M	10.52	7.05	1.04	0.46	0.08	0.264	0.36	1.2
N	38.76	26.50	3.86	1.73	0.16	0.33	0.72	1.5
O	32.78	22.37	3.26	1.46	0.06	0.4026	0.27	1.83
P	1.5	0.50	0.15	0	0.23	0.319	1.04	1.45
Q	3.21	2.14	0.32	0.14	0.08	0.099	0.36	0.45

The susceptible *A. myosuroides* population in the agar bioassay sensitivity test showed a 50% reduction in relative dry matter content at 0.22 pmol a.i. µL^{-1} in V1 and 0.20 pmol a.i. µL^{-1} in V2. The population of F showed an increased ED50 value of 1.37 pmol a.i. µL^{-1} in V1 compared to the ED50 value of 0.55 pmol a.i. µL^{-1} in V2. The calculated RF for population F was 7.12 in V1 and 2.5 in V2. The population J showed a similar response to cinmetyhlin, with 0.79 pmol a.i. µL^{-1} in V1 and 0.27 pmol a.i. µL^{-1} in V2. The calculated RF for population J in the V1 was 14.16 and for V2 1.26. The ED50 values of the other populations for cinmethylin varied between 0.06 pmol a.i. µL^{-1} until 1.35 pmol a.i. µL^{-1} (RF 0.27–2.2) in V1 and 0.08 pmol a.i. µL^{-1} until 0.40 pmol a.i. µL^{-1} (RF 0.4–1.83) for theV2. There were no significant differences for cinmethylin between experiment one and two in the agar bioassay sensitivity test (Table 2.1.4-1).

2.1.4.2. ED50 values and resistance factors – flufenacet

The whole plant pot bioassay for flufenacet resulted in an ED50 value of 23.8 g a.i. ha^{-1} for V1 and 10.5 g a.i. ha^{-1} for V2. The *A. myosuroides* population Q showed a reduction of the ED50 value by 85% in V2 compared to V1. The calculated RF for population Q in V1 was 13.46 and for V2 17.51. The populations N, O and P showed a lower ED50 value in V1 (12.5 g a.i. ha^{-1}, 71.62 g a.i. ha^{-1}, 222.76 g a.i. ha^{-1}) compared to V2 (63.74 g a.i. ha^{-1}, 231.42 g a.i. ha^{-1}, 428.61 g a.i. ha^{-1}). The corresponding RFs for the populations N, O and P were in V1 0.53, 3.01 and 9.36 and in V2 6.07, 22.04 and 40.82. The ED50 values of the other populations for flufenacet varied between 5.1 g a.i. ha^{-1} up to 200.65 g a.i. ha^{-1} (RF 0.22–8.44) in V1 and 4.31 g a.i. ha^{-1} up to 80.43 g a.i. ha^{-1} (RF 0.41–7.66) in V2. There were no significant differences for flufenacet between V1 and V2 in the bioassay test (Table 2.1.4-2).

Table 2.1.4-2: Evaluation of sensitivity of the A. myosuroides populations to flufenacet in a whole plant pot bioassay in greenhouse and agar based resistance test under controlled environmental conditions in a climate chamber. Whereas ED50 = herbicide dosage causing 50% reduction in the relative dry matter content and RF = sensitivity factor. Whole plat pot bioassay V1: lack-of-fit, P = 0.99; V2: lack-of-fit, P = 0.95; Agar test V1: lack-of-fit, P = 0.91; V2: lack-of-fit, P = 0.92.

	Whole Plant Pot Bioassay				Agar Bioassay Sensitivity Test			
Population	**ED50 V1**	**ED50 V2**	**RF V1**	**RF V2**	**ED50 V1**	**ED50 V2**	**RF V1**	**RF V2**
	g a.i. ha^{-1}				**pmol a.i. L^{-1}**			
Sens	23.80	10.50	1.00	1.00	0.04	0.07	1.00	1.00
A	5.65	4.73	0.24	0.45	0.50	0.93	12.92	13.24
B	26.20	22.05	1.10	2.10	0.50	0.74	12.07	10.57
C	5.10	9.35	0.22	0.89	0.60	0.90	13.56	12.86
D	26.35	27.62	1.11	2.63	0.59	0.81	13.22	11.5
E	64.35	38.22	2.70	3.64	0.26	0.30	5.71	4.23
F	200.65	80.43	8.44	7.66	0.04	0.07	0.99	1.02
G	66.55	47.88	3.25	4.56	0.28	0.30	6.34	4.29
H	52.50	38.75	2.21	3.69	0.60	1.02	13.40	14.55
I	0.29	68.67	6.00	6.54	0.05	0.09	1.13	1.25
J	142.45	76.86	6.10	7.32	0.62	0.88	13.40	12.60
K	11.00	5.46	0.46	0.52	0.04	0.00	1.00	1.01
L	7.55	4.31	0.31	0.41	0.05	0.08	1.25	1.20
M	36.51	19.95	1.50	1.90	0.11	0.59	2.75	8.40
N	12.50	63.74	0.53	6.07	0.03	0.45	0.75	6.45
O	71.62	231.42	3.01	22.04	0.47	1.49	11.75	21.30
P	222.76	428.61	9.36	40.82	0.55	2.56	13.75	36.56
Q	320.46	48.195	13.46	17.51	0.10	0.32	2.50	4.59

The susceptible *A. myosuroides* population in the agar bioassay sensitivity test showed for flufenacet a 50% reduction in relative dry matter content at 0.04 pmol a.i. μL^{-1} in V1 and 0.07 pmol a.i. μL^{-1} in V2. The populations of M, N, O and P showed lower ED50 values of 0.11 pmol a.i. μL^{-1}, 0.03 pmol a.i. μL^{-1}, 0.47 pmol a.i. μL^{-1}, 0.55 pmol a.i. μL^{-1} in V1 compared to the ED50 values of 0.59 pmol a.i. μL^{-1}, 0.45 pmol a.i. μL^{-1}; 1.49 pmol a.i. μL^{-1}; 2.56 pmol a.i. μL^{-1} in V2. The calculated RF for the populations M, N, O and P were in V1, 2.75, 0.75, 11.75, 13.75, and in V2 following RFs were calculated as 8.4, 6.45, 21.3, and 36.56. The ED50 values of the other populations for flufenacet varied between 0.04 pmol a.i. μL^{-1} up to 0.62 pmol a.i. μL^{-1} (RF 1–13.4) in V1 and 0.05 pmol a.i. μL^{-1} up to 1.02 pmol a.i. μL^{-1} (RF 1.0–14.55) in V2. There were no significant differences for flufenacet between V1 and V2 in the agar bioassay sensitivity test (Table 2.1.4-2).

2.1.5. DISCUSSION

Pre-emergence testing of herbicides presents numerous challenges. The efficacy of pre-emergence herbicides is affected by soil moisture, soil structure, seed depth, dormancy, germination rate, and time. Their bioavailability also depends on soil properties such as pH, organic matter, and clay content (Beckie *et al.*, 2000). Current whole plant bioassay test systems, especially for pre-emergence herbicides require a large investment of resources (space, manpower) and time. To find a standard method for validating pre-emergence herbicide resistance, this study compared the conventional whole-plant pot bioassay with a newly developed agar bioassay sensitivity test. For this purpose, the two active ingredients flufenacet and cinmethylin were tested on 18 *A. myosuroides* populations. Both active ingredients were carefully selected for this study. Cinmethylin with its new mode of action on which resistance to herbicides can be assumed in the future and flufenacet which has already shown first resistance.

The time required in the recently developed agar bioassay sensitivity test system (12 days) was reduced by 21 days, compared to whole plant pot bioassay (33 days) in the greenhouse (Beckie *et al.*, 2000; Reade & Cobb, 2002). Therefore, hypothesis (i) the agar bioassay sensitivity test is faster than the whole plant pot bioassay can be accepted. In comparison, the Syngenta RISQ test provides valid results within 10 days, but the time of pre-germination of the seeds has to be added (Kaundun *et al.*, 2011). A quick overview of the resistance status is also provided by the Rothamsted Rapid Resistance Test, which delivers results within 14 days (Moss, 1999). Especially, the whole plant pot bioassay requires a lot of time for mixing the soil. Particularly with regard to testing systems dealing with pre-emergence herbicides, the composition of the soil plays a major role. Soil properties such as pH, organic matter content and clay content determine the bioavailability of herbicides (Beckie *et al.*, 2000; Menne *et al.*, 2012a). Therefore, potting mixtures are not suitable for whole plant pot bioassays and it is necessary to mix its own soil. Alternatively, the common soil substrate was exchanged by an agar medium. The exchange was intended to reduce the influence of soil parameters on the herbicide availability. This reduction of environmental factors could not be confirmed by our tests. However, the agar medium did also not increase the reliability of resistance detection. In summary, it can be assumed that the time required for the agar bioassay sensitivity test is restricted to a few very intensive days, while the conventional greenhouse whole plant pot bioassay requires a longer period of time with a lower workload per day.

The results from the whole plant pot bioassay have been reproduced for the majority of the with cinmethylin tested population in the agar bioassay sensitivity test. Nevertheless, hypotheses two needs to be rejected. The discrepancy between the two tests was striking for the populations F, J, K for cinmethylin and was expected of the populations E, G, K, L and N for flufenacet. Almost all RFs from the whole plant pot bioassay are lower in the agar bioassay sensitivity test for cinmethylin, while for flufenacet overall, they increasing with more variation. Same weed-species populations, might express different levels of sensitivity to a specific herbicide. This variation can be investigated in whole plant bioassays described as baseline sensitivity testing before a new herbicide is introduced into the market. Previous attempts already have shown that weed populations can vary greatly in susceptibility to a herbicide and experimental conditions (Ellis & Kay, 1975; Patzoldt *et al.*, 2002). Minor differences in ED50 values between biotypes are naturally occurring (Espeby *et al.*, 2011).Cinmethylin is a new active substance not yet marketed in Europe and never used for the control of *A. myosuroides* (Dayan, 2019). For cinmethylin, resistance factors ranged between one and three with the exception of three biotypes with partly higher resistance factors. Maybe some populations are even more susceptible than the sensitive standard and produce less dry matter in this case. As a result, lower resistance factors were calculated. The population F and J showed reduced susceptibility to cinmethylin within both test systems. Recent studies suggest that selectivity in wheat is both position-dependent but also involves metabolism by cytochrome P450.Thus, if wheat has a CYP 450 that can degrade this herbicide, this or a similar cytochrome could also be present in *A. myosuroides* (Busi *et al.*, 2020).

However, the results do not indicate resistance to cinmethylin. Out of this dataset a baseline sensitivity can be derived. According to the whole-plant pot bioassay in the greenhouse, a dose of 100 g active ingredient (a.i.) ha^{-1} of cinmethyline could be adopted as the baseline. For flufenacet the results of the whole plant pot bioassay in greenhouse could not be reproduced in the agar bioassay sensitivity test. Besides the genetic variability of the population probably the concentration of a.i. and the application of the herbicide could influence the availability of the herbicides. Flufenacet is mainly taken up by the roots, only a small amount is taken up by the leave. In a study of Andreasen *et al.* (2020) they tested an foliar application of flufenacet, after application and complete droplet dry-down, only the crystals of the active ingredients are left on the leaf surface, which makes the herbicide unavailable for foliar uptake (Cobb & Reade, 2011). Within the presented study, herbicide was applicated via the agar plate. It could be possible that a reduced rate of herbicide is taken up by the roots. In contrast to the study of Rosenhauer & Petersen (2015), all plants grew through the agar and came into contact with the herbicide at least with the shoot. In the study of Rosenhauer & Petersen (2015), no clear statement could be made concerning the penetration of the herbicide from the agar into the root zone. In the current study, no clear statement can be made on this either, as the test for cinmethylin gave valid results. Further experiments have to be conducted to test the availability of the herbicide within the root zone.

Only small concentrations are used in the agar bioassay sensitivity test. The influence of the concentration of the a.i. of the herbicide could be a factor. Little is known about this. However, the influence of a.i. concentration on pesticide uptake has been systematically studied only for glyphosate. It is now known that glyphosate uptake is closely related to its concentration in the spraying solution. The higher the a.i. concentration, the greater the uptake (Cranmer & Linscott, 1991). This may also have an influence on the results in the agar bioassay sensitivity test. On the other hand, only fragmentary information is available for some other chemicals like cinmethylin or flufenacet.

In contrast to the F, J and Q populations, the A, C, D and H populations showed a lower resistance factor in the agar bioassay sensitivity test compared to the whole plant pot bioassay in the greenhouse. It is possible that the herbicide was metabolized more quickly here due to higher temperatures in the greenhouse, which reduces the herbicidal effect on the plant. This phenomenon has already been observed in other trials were post-emergence herbicide were used (Varanasi *et al.*, 2016a). This could result in a misinterpretation of the resistance factors in the whole plant pot bioassay trials. In order to draw reliable and precise results from the resistance test, it is important to ensure that the external factors, like water supply and temperature are standardized and not subject to any fluctuations. The results of this study may not be transferable to other *A. myosuroides* populations, but provide information on the susceptibility status in the area where the biotypes used originate (Espeby *et al.*, 2011). However, while reduced sensitivity or resistance can often be demonstrated in the laboratory, this does not necessarily mean that weed control under field conditions will no longer take place. The term 'practical resistance' has been established, which describes the loss of weed control under field conditions due to a shift in sensitivity (Eppo, 2015). To interpret the results correctly, it is necessary to consider the resistance factors and the actual rate required to control the resistant population (Heap, 1994). The calculated resistance factor depends on the sensitive standard used. The sensitivity of the biotypes was higher against cinmethylin, especially in the agar bioassay sensitivity test, than with flufenacet, which has been used for many years. In conclusion, it can be confirmed that the new agar bioassay sensitivity test has high potential, to increase the test volume of samples due to the shorter test duration. Nevertheless, additional tests should be carried out to clearly confirm resistance. Especially, to increase the validity of the agar bioassay sensitivity test, further testing with more populations and pre-emergence herbicides should be conducted.

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2.1.6. REFERNCES

BAILLY GC, DALE RP, ARCHER SA, WRIGHT DJ & KAUNDUN SS (2012) Role of residual herbicides for the management of multiple herbicide resistance to ACCase and ALS inhibitors in a black-grass population. *Crop Protection* **34,** 96–103.

BASF (2018) BASF reicht Zulassungsdossiers für zwei neue Herbizid-Wirkstoffe ein. https://www.basf.com/global/de/media/news-releases/2018/06/p-18-242.html., last accessed 14 Dec 2020.

BECKIE HJ, HEAP IM, SMEDA RJ & HALL LM (2000) Screening for Herbicide Resistance in Weeds 1. *Weed Technology* **14,** 428–445.

BOUTSALIS P (2001) Syngenta Quick-Test. A Rapid Whole-Plant Test for Herbicide Resistance 1. *Weed Technology* **15,** 257–263.

BUSI R (2014) Resistance to herbicides inhibiting the biosynthesis of very-long-chain fatty acids. *Pest management science* **70,** 1378–1384.

CAMPE R, HOLLENBACH E, KÄMMERER L et al. (2018) A new herbicidal site of action: Cinmethylin binds to acyl-ACP thioesterase and inhibits plant fatty acid biosynthesis. *Pesticide Biochemistry and Physiology* **148,** 116–125.

CHAUVEL B, GUILLEMIN J-P & COLBACH N (2009) Evolution of a herbicide-resistant population of Alopecurus myosuroides Huds. in a long-term cropping system experiment. *Crop Protection* **28.,** 343–349.

DAYAN FE (2003) Current Status and Future Prospects in Herbicide Discovery. *Plants (Basel, Switzerland)* **8,** 341.

DAYAN FE, ROMAGNI JG & DUKE SO (2003) Herbicides, Cinmethylin. American Cancer Society.

DÜCKER R, ZÖLLNER P, PARCHARIDOU E, RIES S, LORENTZ L & BEFFA R (2019) Enhanced metabolism causes reduced flufenacet sensitivity in black-grass (Alopecurus myosuroides Huds.) field populations. *Pest management science* **75,** 2996–3004.

ELLIS M & KAY QON (1975) Genetic variation in herbicide resistance in scentless mayweed (Tripleurospermum inodorum (L.) Schultz Bip.) I. Differences between populations in response to MCPA. *Weed Research* **15,** 307–315.

EPPO (2015) PP 1/213 (4) Resistance risk analysis. *EPPO Bulletin* **45,** 371–387.

ESPEBY LÅ, FOGELFORS H & MILBERG P (2011) Susceptibility variation to new and established herbicides: Examples of inter-population sensitivity of grass weeds. *Crop Protection* **30,** 429–435.

GRESSEL J & SEGEL LA (1990) Modelling the Effectiveness of Herbicide Rotations and Mixtures as Strategies to Delay or Preclude Resistance. *Weed Technology* **4,** 186–198.

HEAP I (2014) Global perspective of herbicide-resistant weeds. *Pest management science* **70,** 1306–1315.

HEAP I (2020) International Survey of Herbicide Resistant Weeds. http://weedscience.com/, last accessed 18 Jan 2020.

HEAP IM (1994) Identification and documentation of herbicide resistance. *Phytoprotection* **75,** 85–90.

HULL RI & MOSS SR (2012) Is the increasing reliance on residual herbicides for Alopecurus myosuroides (black-grass) control sustainable? *Aspects of Applied Biology,* 25–32.

KAUNDUN SS, HUTCHINGS S-J, DALE RP, BAILLY GC & GLANFIELD P (2011) Syngenta 'RISQ' test. A novel in-season method for detecting resistance to post-emergence ACCase and ALS inhibitor herbicides in grass weeds. *Weed Research* **51,** 284–293.

KNEZEVIC SZ, STREIBIG JC & RITZ C (2007) Utilizing R Software Package for Dose-Response Studies. The Concept and Data Analysis. *Weed Technology* **21,** 840–848.

LETOUZÉ & GASQUEZ (2000) A pollen test to detect ACCase target-site resistance within Alopecurus myosuroides populations. *Weed Research* **40,** 151–162.

LUTMAN PJW, MOSS SR, COOK S & WELHAM SJ (2013) A review of the effects of crop agronomy on the management of Alopecurus myosuroides. *Weed Research* **53,** 299–313.

MENNE HJ & HOGREFE C, eds (2012) Impact of multiple resistance mechanisms in black-grass (Alopecurus myosuroides Huds.) populations on the of cereal herbicides. Proceedings, 25th German Conference on Weed Biology and Weed Control. Braunschweig, Germany.

MENNE HJ, LABER B, KERLEN D & BEFFA R, eds (2012) Effectiveness of flufenacet in controlling resistant black-grass (Alopecurus myosuroides Huds.) - comparison of glasshouse and field trial results. Proceedings, 25th German Conference on Weed Biology and Weed Control. Braunschweig, Germany.

MOSS S (1999) The "Rothamsted rapid resistance test" for detecting herbicide - resistance in annual grass - weeds., last accessed 18 Jan 2020.

MURRAY BG, FRIESEN LF, BEAULIEU KJ & MORRISON IN (1996) A Seed Bioassay to Identify Acetyl-CoA Carboxylase Inhibitor Resistant Wild Oat (Avena fatua) Populations. *Weed Technology* **10,** 85–89.

PATZOLDT WL, TRANEL PJ & HAGER AG (2002) Variable herbicide responses among Illinois waterhemp (Amaranthus rudis and A. tuberculatus) populations. *Crop Protection* **21,** 707–712.

READE JPH & COBB AH (2002) New, quick tests for herbicide resistance in black-grass (Alopecurus myosuroides Huds) based on increased glutathione S-transferase activity and abundance. *Pest management science* **58,** 26–32.

RÜEGG WT, QUADRANTI M & ZOSCHKE A (2007) Herbicide research and development. Challenges and opportunities. *Weed Research* **47,** 271–275.

STREIBIG JC (1988) Herbicide bioassay. *Weed Research* **28,** 479–484.

ULBER L, NORDMEYER H & ZWERGER P (2013) Resistance risk assessment within herbicide authorisation--a call for sensitivity data. *Pest management science* **69,** 160–164.

VARANASI A, PRASAD PV & JUGULAM M (2016) Chapter Three - Impact of Climate Change Factors on Weeds and Herbicide Efficacy. In: *Advances in Agronomy*, Vol. 135 (ed. DL SPARKS), 107–146. Academic Press.

2.2. Weed Suppressive Ability of Cover Crop Mixtures Compared to Repeated Stubble Tillage and Glyphosate Treatments

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2.2.1. ABSTRACT

The utilization of an effective stubble management practice can reduce weed infestation before and in the following main crop. Different strategies can be used, incorporating mechanical, biological, and chemical measures. This study aims at estimating the effects of cover crop (CC) mixtures, various stubble tillage methods, and glyphosate treatments on black-grass, volunteer wheat and total weed infestation. Two experimental trials were conducted in Southwestern Germany including seven weed management treatments: flat soil tillage, deep soil tillage, ploughing, single glyphosate application, dual glyphosate application, and a CC mixture sown in a mulch-till and no-till system. An untreated control treatment without any processing was also included. Weed species were identified and counted once per month from October until December. The CC mixtures achieved a black-grass control efficacy of up to 100%, whereas stubble tillage and the single glyphosate treatment did not reduce the black-grass population, on the contrary it induced an increase of black-grass plants. The dual glyphosate application showed, similar to the CC treatments, best results for total weed and volunteer wheat reduction. The results demonstrated, that well developed CCs have a great ability for weed control and highlight that soil conservation systems do not have to rely on chemical weed control practices.

KEYWORDS

biological; black-grass (*Alopecurus myosuroides* Huds.), chemical; mechanical; mulch-till; no-till systems; stubble tillage; weed management

2.2.2. INTRODUCTION

A crop rotation, including spring crops, requires an effective weed management strategy during the crop-free period. This might include biological, mechanical, and chemical (also repeated and in combination) weed control tools on the fallow ground not in production in autumn. These tools have the aim to encourage the germination of volunteer crops, remove emerged weeds, reduce available sources, especially for perennial weeds, and to avoid a new weed seed production. The success of a weed management technique during the crop-free period may have a major impact on the weed seed bank, and weed infestation on the subsequent crops. Weeds compete for resources with the main crops and may also act as a host for pests and diseases (Norris and Kogan, 2000). The ergot fungus (*Claviceps purpurea* (Fr.) Tul.) for example uses *Alopecurus myosuroides* Huds. (*A. myosuroides*) as an alternate host (Mantle and Shaw, 1976). An effective weed control strategy therefore improves plant health and provides yield stability. The application of synthetic herbicides is a common weed control practice in conventional farming systems. The use of non-selective herbicides (e.g., glyphosate) is a non-time-intense and efficient weed management practice particularly in conservation agriculture systems. *A. myosuroides*, an annual grassy-weed (Poaceae), became a major problem in autumn sown crops in Western Europe (Moss, 2017). The increasing impact of *A. myosuroides* in agricultural cropping systems can be attributed to the modifications on the current agricultural strategies, like increasing numbers of autumn sown crops, the alteration of cropping and tillage systems and the consequent usage of herbicides with the same mode of action (Moss, 2017). Several weed species have developed resistance to herbicides including glyphosate (Powles and Yu, 2010). Since *A. myosuroides* has already evolved field resistance to multiple herbicide modes of action (Heap, 2017), increasing the reliance on glyphosate can lead to a resistance to it (Davies and Neve, 2017). The current public concern raised, regarding the use of glyphosate in agriculture and the restrictions enforced in different countries, increases the necessity to search for alternative measures and different weed management tools. Biological and mechanical control methods might be an option to compete with resistant populations as well as to mitigate the development of herbicide resistant weeds.

Mechanical weed control practices, including tillage, might differ regarding the implementation, timing, and frequency (Pekrun and Claupein, 2006). This might include flat tillage (<5 cm) and as well a deep stubble tillage (>5 cm) (Melander et al., 2017). Ploughing buries the weed seeds and mostly prevents them to emerge from deeper soil layers. Systems with a lower or superficial soil disturbance, compared to ploughing, usually result in a greater weed infestation (Wrucke and Arnold, 1985) and weed seed accumulation near the soil surface (Colbach et al., 2006). However, reduced tillage systems have the advantage of decreasing run-off, increasing aggregate stability (Hernanz et al., 2002) and preserving a higher soil moisture (Vita et al., 2007). Repeated flat or medium deep tillage may combine the benefits of reduced tillage systems for soil conservation with a sufficient weed control, yet with a possible negative impact concerning nutrient losses, soil compaction, or carbon gas emissions.

Winter cover crops (CCs), used as a biological weed control measure (Snapp et al., 2005), may demonstrate several advantages, including nutrient recycling efficiency (Snapp et al., 2005) and reduced soil erosion (Langdale et al., 1991). The success of CCs as an integrated weed management practice, is related to a fast emergence and high soil cover, which depends on the chosen species, soil properties, and the weather conditions at the field location. Using different cover crop (CC) species within a mixture increases the resilience for management failures, bad weather conditions, and combines species-specific benefits (Wortman et al., 2012). Seed predation, which may also act as a biological weed control measure (Hartwig and Ammon, 2002), is enhanced in cover-cropping (Blubaugh et al., 2016) and no-till systems (Petit et al., 2017) and decreases the amount of weed seeds at the soil surface. The straw management, also in combination with the different weed management practices as mentioned above, has an impact on weed infestation. Generally, straw disposal can for example reduce the number of *A. myosuroides* plants, due to weed seed removal from the field (Moss, 1979). In no-till systems the straw surface coverage, which generates a physical barrier, is reducing the weed density (Bilalis et al., 2003). Otherwise, the herbicide efficacy could be reduced by crop residues (Dao, 1991). On the other hand, the presence of straw in CC systems might lead to an immobilization of nitrogen, which will then narrow the CC development and their subsequent success for weed suppression (Kahnt, 1983).

There is little information available about the potential of repeated flat and deep stubble tillage in comparison to ploughing and cover-cropping to substitute herbicide applications in autumn. In a non-inversion tillage system grass weeds, like *A. myosuroides*, might be encouraged (Froud-Williams et al., 1984). Furthermore, CCs are a suitable tool for broad-leave weed control (Teasdale, 1996). Within cover-cropping systems grass weeds may also become a severe challenge (Clements et al., 2000) which might require the use of herbicides (Teasdale, 1996). The presence and absence of straw will additionally deliver information about the impact of straw management in combination with different weed management treatments on weed infestation. This study aims at estimating the ability of selected biological, mechanical, and chemical weed control practices on weed suppression before spring cropping. The following hypotheses were investigated: (i) stubble tillage and CCs have similar success in reducing weeds as glyphosate applications; (ii) repeated stubble tillage is a more effective weed suppression measure in comparison to a single deep, turning soil tillage; (iii) the sowing method of CCs (mulch-tillage and no-tillage) has an impact on the success of weed suppression; (iv) the removal of straw after harvest is influencing the weed infestation.

The study was implemented at field sites with an increased population of *A. myosuroides*. The results may clarify if tillage, herbicide application, or cover-cropping can reduce the number of *A. myosuroides* plants. CCs were sown within a mulch-till and no-till systems to evaluate if no-till systems lead to an increasing number of weeds in comparison to stubble tillage systems as shown by Gruber et al. (2012).

2.2.3. MATERIALS AND METHODS

2.2.3.1. Experimental Sites

Two field experiments (Binsen: 48°25´22.0´´ N 8°53´15.4´´ E and Risp: 48°25´06.3´´ N 8°53´48.0´´ E) were conducted in Southwestern Germany from August until December 2017. The weather data are shown in Table 2.2.3-1.

Table 2.2.3-1: Monthly minimum (Min.), maximum (Max.), average temperature (T) and precipitation at Southwest-Germany for July until December 2017.

	Min. T (°C)	**Max. T (°C)**	**Average T (°C)**	**Precipitation (mm)**
July	12.6	24.9	18.5	119.5
August	12.2	24.6	18.3	88.2
September	7.0	17.9	12.0	35.3
October	4.7	15.8	9.7	40.1
November	0.7	6.8	3.6	76.0
December	-1.3	3.6	1.2	55.9

The soil type at both trials was characterized as a loamy silt with pH values of 6.9 (field Binsen) and 5.9 (field Risp). The fields had a different crop rotation history with the same previous crop at the beginning of the experiment. Crop rotation at the field Binsen was winter wheat (2013), triticale (2014), spring barley (2015), peas (2016), and winter wheat (2017). The trial at the field Risp had a crop rotation of peas (2013), winter wheat (2014), red clover (2015), flowering mixture (2016), followed by winter wheat (2017). The winter wheat was harvested at the 1st of August at both trials. The experimental trials were set up as a randomized strip-plot design. The two factorial experiments included seven weed management practices with regard to mechanical, chemical, and biological treatments (1st factor). The untreated control plots were left without any weed control treatment. The details according to the weed management treatments are shown in Table 2.2.3-2. The 2nd factor (which was implemented as the strip) combined the same weed management treatments as mentioned before including the presence and absence of straw. The straw from the plots with the straw removal was baled and taken from the plots at the same day as the harvest. In total, 16 treatments with 3 repetitions were included at both field trials.

The plots had a size of 16.5 x 5 m (field Binsen) and 21.5 x 5 m (field Risp). The CC mixture sown at both trials for treatments 7 and 8 was provided by DSV-Saaten (Deutsche Saatveredelung AG, 2018) and included the following CC species (their ratios within the mixture are shown in brackets): *Avena strigosa* Schreb. (45%), *Fagopyrum esculentum* Moench (18%), *Linum usitatissimum* L. (12%), *Phacelia tanacetifolia* Benth. (6 %), *Raphanus sativus* var. *oleiformis* (6%), *Sinapis alba* L. (6%), *Brassica carinata* A.Braun (4%), *Helianthus annuus* L. (2%), *Camelina sativa* Crantz (1%). The plots with the CC treatments sown with mulch-till (treatment 7) were prepared with a cultivator and a rotary harrow. A Cambridge roller was used after sowing to increase the soil contact of the seeds and to improve the CC seed germination.

Table 2.2.3-2: Weed management treatments, weed control type and treatment dates for the experimental field sites at Binsen and Risp. Weed management dates include dates for tillage, herbicide applications, and sowing dates for the cover crop mixtures. (DAH = Days after harvest).

Treatment[1]		Weed management practices (depth in cm / concentration in l ha^{-1} / seed density kg ha^{-1})	Weed control type	Weed management (date)	Weed management (DAH)
1	Control	Weed fallow without weed management	-	-	-
2	FST	Flat soil tillage with rotary harrow (5 cm)	mechanical	8. August, 6. September, 14. October	8, 37, 75
3	DST	Deep soil tillage with wing share cultivator (15-16 cm)	mechanical	8. August, 6. September, 15. October	8, 37, 76

4	PL	Turning soil tillage with plough (25 cm)	mechanical	14. August	14
5	GLY	Single Glyphosate treatment (4 l ha^{-1})	chemical	6. September	37
6	GLY+GLY	Dual Glyphosate treatment (4 l ha^{-1})	chemical	6. September, 4. October	37, 75
7	CC+MT	Cover crop mixture + mulch-till (1-1.5 cm, 25 kg ha^{-1})	biological	19. August	19
8	CC+NT	Cover crop mixture + no-till (1-1.5 cm, 25 kg ha^{-1})	biological	7. August	7

[1] Flat soil tillage (FST), deep soil tillage (DST), ploughing (PL), single glyphosate application (GLY), dual glyphosate application (GLY+GLY), cover crop mixture + mulch-till (CC+MT) and cover crop mixture + no-till (CC+NT).

2.2.3.2. Data Collection

Individual weed species as well as the total amount of plants were identified and counted at three dates: 12th of October (73 DAH), 17th of November (109 DAH) and 13th of December (135 DAH). This was performed with a circular 0.33 m^2 frame at four randomly chosen spots per plot. CC biomass was cut at both mulch-till sown and no-till treatments once at the 14th of October. The CC biomass was measured to determine which sowing technique results in a greater CC development. The biomass of 0.33 m^2 was cut and fresh weed and CC biomass measured at four randomly chosen locations per plot.

2.2.3.3. Data Analysis

RStudio software (Version 1.1.453, RStudio Team, Boston, MA, USA) was used for analyzing the data. Prior to analysis, the data was visually checked for normal distribution and homogeneity of variance. A transformation of the data was not necessary before doing an analysis of variance (ANOVA). The Tukey-HSD test ($p \leq 0.05$) was performed to compare the means of the different treatments. The weed control efficacy (WCE), *A. myosuroides* control efficacy (ACE), and volunteer wheat control efficacy (VCE) was calculated according to Rasmussen (1991) and Machleb et al. (2018):

$$\text{WCE, ACE, VCE (\%)} = 100 - wt\ (0.01 \times wc)^{-1} \qquad (1)$$

whereby wt is the weed, *A. myosuroides* or volunteer wheat density (weeds m^{-2}) of the weed management treatments, and wc is the weed, *A. myosuroides* or volunteer wheat density (weeds m^{-2}) of the untreated control plots.

2.2.4. RESULTS

2.2.4.1. Total Weed Suppression

Even though a diverse crop rotation was conducted at both experimental field sites, including winter and spring crops, *A. myosuroides* was the most dominant monocotyledons weed species, besides volunteer wheat. Other than that, dicotyledons like *Lamium purpureum* L., *Veronica persica* Poir., *Stellaria media* Vill., *Thalaspi arvense* L., and *Raphanus raphanistrum* L. were the dominant weed species (Table 2.2.4-4). The untreated control plots at the field Binsen showed a mean weed infestation of 96.9 weeds m^{-2} (averaged over all counting dates). The WCE of all soil tillage treatments (flat soil tillage (FST), deep soil tillage (DST) and ploughing (PL) at 73 DAH was between 1-76%, which was significantly lower (Figure 2.2.4-1) than for both glyphosate and the CC treatments. The FST and DST treatments showed an improved WCE with up to 82% at 109 and 135 DAH. Nevertheless, repeated tillage (FSL, DST) treatments resulted in lower WCE than the CC and the GLY+GLY (dual glyphosate application) treatments throughout the season. The CC+NT (cover crop mixture + no-till) treatment showed a WCE of 88% (135 DAH). The GLY+GLY treatment showed the highest WCE with more than 97% (109 DAH).

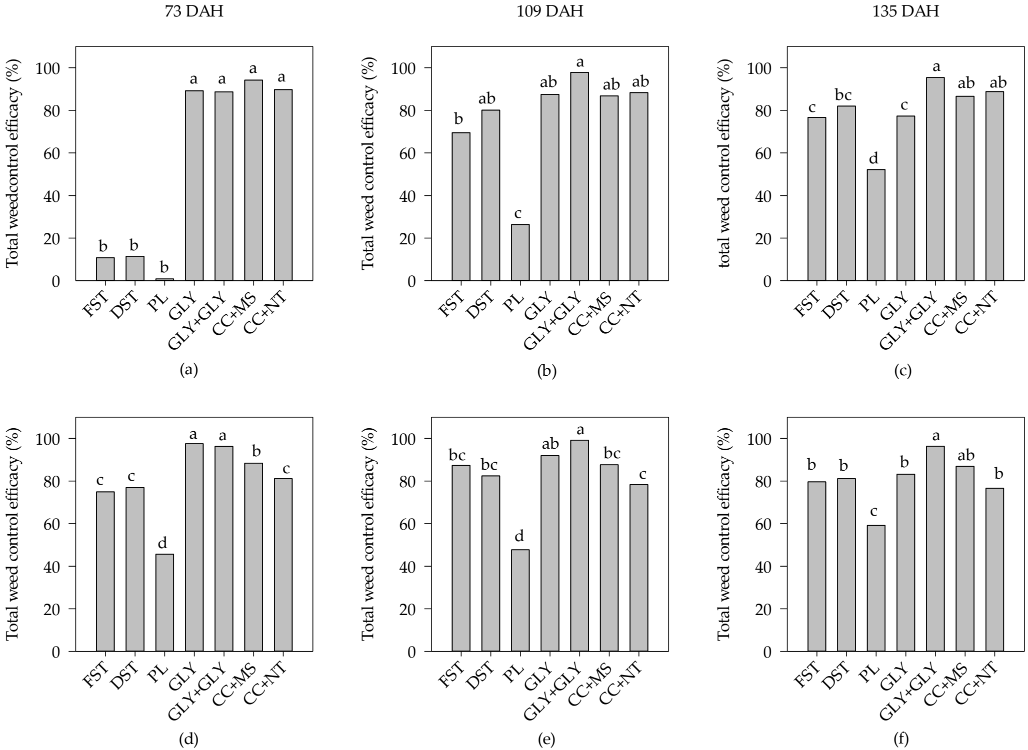

Figure 2.2.4-1: Average total weed control efficacy of the treatments flat soil tillage (FST), deep soil tillage (DST), ploughing (PL), single glyphosate application (GLY), dual glyphosate application (GLY+GLY), cover crop mixture + mulch-till (CC+MT), and cover crop mixture + no-till (CC+NT) at the two trials at the fields (a-c) Binsen and (d-f) Risp. (a,d) 73, (b,e) 109, and (c,f) 135 days after harvest (DAH). Different small letters within one graph show significant differences according to Tukey-HSD test ($p \leq 0.05$). Means with identical letters do not differ significantly.

The untreated control plots at the trial at field Risp showed a generally higher mean weed infestation of 183.7 weeds m^{-2} (averaged over all counting dates). Similar to the trial at the field Binsen the GLY (single glyphosate application) and GLY+GLY performed significantly best, with a WCE of approximately 97% 73 DAH. Whereby the dual glyphosate application (GLY+GLY) increased the WCE 135 DAH up to 99%, the single treatment (GLY) reduced the WCE and showed no significant differences according to the WCE, compared to the CC and the repeated tillage (FST, DST) treatments 135 DAH. The CC+NT treatments seem to reduce weeds less efficient than the CC+MT (cover crop mixture + mulch-till) treatments. This trend was only significant at the field site at Risp 73 DAH. The PL treatments performed always significantly worse, at both trials (excluding the field Binsen 73 DAH), resulting in a WCE reaching a maximum of 59% 135 DAH at the field site at Risp. The factor straw was not significant, therefore Figure 2.2.4-1 is giving average values for total WCE, including treatments with straw and those with straw removal.

2.2.4.2. *A. myosuroides* Suppression

The untreated control at the field Binsen showed an average number of *A. myosuroides* with 7.1 plants m^{-2} (73, 109, and 135 DAH). Whereas all weed control practices were able to reduce the total amount of weeds, compared to the control, treatments like FST and DST increased the number of *A. myosuroides* to 20.6 and 18.7 plants m^{-2} (73, 109, and 135 DAH at field Binsen), respectively. The control treatments at the field Risp had a higher amount of *A. myosuroides* with 8.6 plants m^{-2}. At both trials the repeated stubble tillage (FST, DST) and the PL treatment achieved a significant increase of *A. myosuroides* 73 DAH (Table 2.2.4-1). The CC treatments showed the highest ACE from 91.7 up to 100%. Both glyphosate treatments (GLY and GLY+GLY) are not showing the same efficacy against *A.*

myosuroides as for the total weed control. The presence of straw reduced the number of *A. myosuroides* plants significantly within the FST and the DST treatments at the field Binsen.

Table 2.2.4-1: Average Alopecurus myosuroides Huds. control efficacy of flat soil tillage (FST), deep soil tillage (DST), ploughing (PL), single glyphosate application (GLY), dual glyphosate application (GLY+GLY), cover crop mixture + mulch-till (CC+MT) and crop mixture + no-till (CC+NT) treatments in combination with the presence (+) or absence (-) of straw 73 days after harvest (DAH) at the two trials at the fields Binsen and Risp. Different small letters within one column show significant differences according to Tukey-HSD test ($p \leq 0.05$). Means with identical letters do not differ significantly. Different capital letters show significant differences within one experiment and within one treatment according to the presence or absence of straw. Means with no capital letter do not differ according to the Tukey-HSD test ($p \leq 0.05$).

	73 DAH			
	Binsen		Risp	
Treatment	- straw	+ straw	- straw	+ straw
FST	-603.5 ^{cB}	-244.5 ^{bA}	-279.0 ^{d}	-299.0 ^{cd}
DST	-860.6 ^{dB}	-337.7 ^{bA}	-114.0 ^{bcd}	-500.4 ^{d}
PL	-356.4 ^{b}	-230.4 ^{b}	-198.1 ^{cd}	-185.9 ^{bcd}
GLY	-33.0 ^{a}	45.2 ^{a}	28.1 ^{ab}	24.8 ^{ab}
GLY + GLY	-31.6 ^{a}	33.3 ^{a}	6.7 ^{abc}	-45.5 ^{abc}
CC + MT	100.0 ^{a}	100.0 ^{a}	100.0 ^{a}	97.4 ^{a}
CC + NT	100.0 ^{a}	95.8 ^{a}	91.7 ^{a}	94.1 ^{a}

The factor straw had a significant effect on the suppression of *A. myosuroides* at the field Risp 109 DAH within the DST and the GLY treatments (Table 2.2.4-2). The other treatments at field Risp and also at field Binsen were not affected by the presence or absence of straw. At both trials 109 DAH the GLY+GLY treatment showed an ACE up to 80.8%. Whereby, the GLY treatment was increasing the amount of *A. myosuroides* compared to the control, which resulted in a minimum ACE of -119.8% (field Risp). The CC treatments again performed best with an ACE between 94.4-100% (both trials). Both CC treatments show an ACE of 100% at both field trials 135 DAH (Figure 2.2.4-2). FST and DST treatments increased the amount of *A. myosuroides*, compared to the control. ACE for the FST treatment was -175.0% at the field Binsen and -54.7% at the field Risp (135 DAH). The GLY treatment was also inducing an increase of *A. myosuroides* plants compared to the control, which showed an ACE of -262.5% (field Binsen). The GLY+GLY treatment showed an ACE up to 52.2% (field Risp). The absence or presence of straw had no significant effect on the ACE at both trials 135 DAH.

Table 2.2.4-2. Average Alopecurus myosuroides Huds. control efficacy of flat soil tillage (FST), deep soil tillage (DST), ploughing (PL), single glyphosate application (GLY), dual glyphosate application (GLY+GLY), cover crop mixture + mulch-till (CC+MT) and crop mixture + no-till (CC+NT) treatments in combination with the presence (+) or absence (-) of straw 109 days after harvest (DAH) for the two trials at the fields Binsen and Risp. Different small letters within one column show significant differences according to Tukey-HSD test ($p \leq 0.05$). Means with identical letters do not differ significantly. Different capital letters show significant differences within one experiment and within one treatment according to the presence or absence of straw. Means with no capital letter do not differ according to Tukey-HSD test.

	109 DAH			
	Binsen		Risp	
	- straw	+ straw	- straw	+ straw
FST	-19.0 ^{a}	-6.7 ^{a}	47.4 ^{abc}	21.5 ^{bc}
DST	-1.4 ^{a}	-3.4 ^{a}	13.3 ^{bcA}	-40.7 ^{cB}
PL	-160.2 ^{b}	-182.5 ^{b}	-14.1 ^{c}	-44.1 ^{c}
GLY	-13.0 ^{a}	-4.2 ^{a}	-119.8 ^{dB}	-46.3 ^{cA}
GLY + GLY	75.5 ^{a}	60.0 ^{a}	80.8 ^{ab}	64.4 ^{ab}
CC + MT	100.0 ^{a}	96.7 ^{a}	100.0 ^{a}	100 ^{a}
CC + NT	94.4 ^{a}	100.0 ^{a}	96.7 ^{a}	96.7 ^{ab}

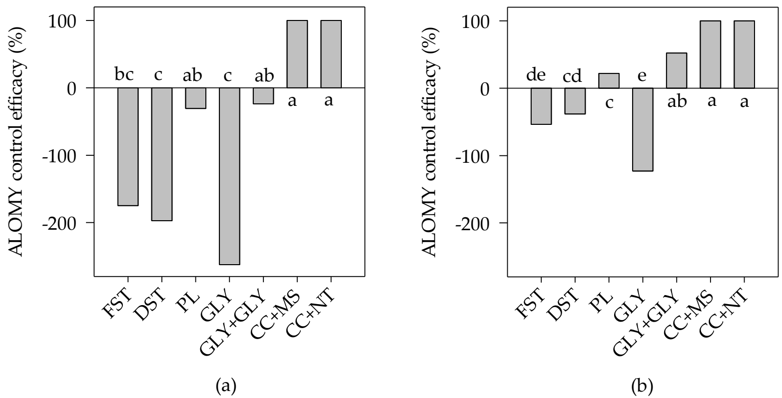

Figure 2.2.4-2: Average Alopecurus myosuroides Huds. (A. myosuroides) control efficacy of flat soil tillage (FST), deep soil tillage (DST), ploughing (PL), single glyphosate application (GLY), dual glyphosate application (GLY + GLY), cover crop mixture + mulch-till (CC+MT) and crop mixture + no-till (CC+NT) treatments at the fields (a) Binsen and (b) Risp 135 days after harvest. Different small letters within one graph show significant differences according to the Tukey-HSD test ($p \leq 0.05$). Means with identical letters do not differ significantly.

2.2.4.3. Volunteer Wheat Suppression

Volunteer wheat was the main weed within both trials. The amount of volunteer wheat achieved 72.9 (field Binsen) and 138.6 plants m^{-2} (field Risp), averaged over the three counting dates. At both trials and all counting dates, the GLY+GLY treatments had a VCE of 100% (Figure 2.2.4-3). The GLY and CC+MT treatments achieved similar results with a VCE of 99.2% at field Binsen 135 DAH. The VCE at the field Risp for the GLY and CC+MT treatments were only slightly lower with 96.1 and 98.1%, respectively (135 DAH). The CC+NT treatments, especially at the trial at field Risp, showed significantly less VCE, compared to both glyphosate (GLY and GLY+GLY) and the CC+MT treatments. Generally, all treatments were able to reduce the amount of volunteer wheat and reached at least 84.4% VCE. The absence or presence of straw had no significant effect according to VCE at both trials (73, 109, and 135 DAH).

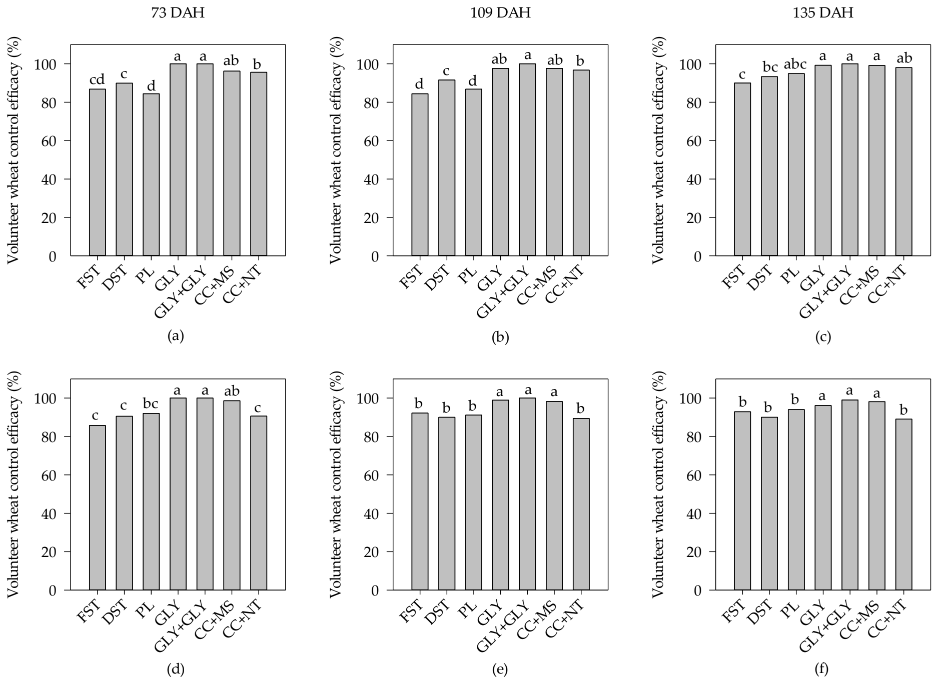

Figure 2.2.4-3: Average volunteer wheat control efficacy of flat soil tillage (FST), deep soil tillage (DST), ploughing (PL), single glyphosate application (GLY), dual glyphosate application (GLY + GLY), cover crop mixture + mulch-till (CC+MT) and crop mixture + no-till (CC+NT) treatments at the two trials at the fields (a-c) Binsen and (d-f) Risp. (a,d) 73, (b,e) 109 and (c,f) 135 days after harvest (DAH). Different small letters within one graph show significant differences according to the Tukey-HSD test ($p \leq 0.05$). Means with identical letters do not differ significantly.

2.2.4.4. CC Biomass

Even though the CC+NT treatment showed in some cases significantly less WCE and VCE, compared to the CC+MT treatment, the fresh CC biomass was not significantly different between those two treatments (Table 2.2.4-3). The factor straw had no statistical impact on the fresh CC biomass. The CC+MT treatment with the presence of straw at the field Binsen and Risp had a fresh CC biomass of 26.9 and 30.5 t ha^{-1}, respectively. The CC+NT treatments, also with straw, showed fresh biomass values of 25.9 (field Binsen) and 27.3 t ha^{-1} (field Risp). Neither the sowing technique (no-till or mulch-till) of CCs, nor the presence or absence of straw had an impact on the fresh weight of weeds.

*Table 2.2.4-3**:** The fresh cover crop biomass (t ha^{-1}) 8 (CC+NT) and 9 (CC+MT) weeks after sowing for the trials at the fields Binsen and Risp. CC+MT = cover crop mixture + mulch-till. CC+NT = cover crop mixture + no-till. - straw = straw removal after harvest. + straw = no straw removal from the previous crop. n.s. = means do not differ significantly within one experiment based on the Tukey-HSD test ($p \leq 0.05$).*

Treatments		Cover crop fresh weight	
		Binsen	Risp
CC+MT	- straw	32.0 $^{n.s.}$ (2.8)	28.2 $^{n.s.}$ (4.0)
	+ straw	26.9 $^{n.s.}$ (3.8)	30.5 $^{n.s.}$ (9.3)
CC+NT	- straw	33.1 $^{n.s.}$ (0.9)	29.0 $^{n.s.}$ (8.9)
	+ straw	25.9 $^{n.s.}$ (3.6)	27.3 $^{n.s.}$ (4.5)

2.2.5. DISCUSSION

Stubble management can have a big impact on weed dynamics (Melander et al., 2013). The result of postharvest tillage on annual weeds mainly relates to the weed flora, the seed bank, and the dormancy status of the seeds (Melander et al., 2013). Flat postharvest tillage incorporates crop residues and stimulates volunteer wheat to germinate. Multiple soil tillage induces weed seeds for germination and destroys and buries them at the subsequent tillage. This might decrease the total weed seed amount in the soil.

No clear differences concerning weed suppression were found between flat (FST) and deep stubble (DST) cultivation, which had also been demonstrated by Boström (1999). In the past, the shallow plough, as stubble tillage practise, was seen as most effective tool for weed management in Germany and Austria, as reported by Gruber et al. (2012). Within our study ploughing (PL) showed worst results concerning WCE among all treatments. It is therefore not reasonable to recommend a deep soil tillage (including ploughing), which is labor intense and costly and does not provide benefits for weed control and soil conservation. In this study, ploughing was done early after harvest. However, a late treatment before weed seed maturity might improve the performance. The generally moderate performance of all mechanical treatments in comparison with the chemical and the biological treatments might be caused by the wet weather conditions during autumn. Cirujeda and Taberner (2006) who harrowed in cereals and state that a high WCE of harrowing is attributed to dry conditions after harrowing. From time to time inversion tillage or stubble management might be useful in order to control weeds in highly infested fields (Gruber et al., 2012). Ploughing, especially in combination with stubble tillage (Melander et al., 2012), is a useful tool against perennial and root spreading weeds. At both field trials, annual weeds were dominant, which allows a reduction of the tillage intensity Gruber and Claupein (2009) and a conservation stubble management with reduced soil disturbance. Pekrun and Claupein (2006) recommend to leave the stubble undisturbed. In terms of a biological weed control strategy, keeping the freshly produced weed seeds on the soil surface enhances biodiversity and increases seed predation as biological weed control (Westerman et al., 2003). The experiments had shown that both CC treatments (CC+NT and CC+MT) achieved an effective weed control during the crop-free period from August until December. In contrast to Brust et al. (2011; 2014) CCs were also able to suppress volunteer wheat. Especially *A. myosuroides*, which tend to be the most challenging grass weed, was successfully controlled by CCs, whereas stubble tillage and glyphosate application mostly failed this effect. The CC treatments reached an ACE up to 100% and a WCE up to 94%. The weed suppression potential of CCs has been proven by several studies (Brust, Claupein et al., 2014; Kunz et al., 2016; Melander et al., 2013). Winter CC cultivation has the potential to shift the use of herbicides towards a postemergence herbicide program (Teasdale, 1996). Weed seed germination and establishment is reduced in cover-cropping systems, but the amount of weed seeds in the soil may increase in the upper layer, especially in no-tillage systems. The success of CCs concerning their WCE is site specific and relates to the CC chosen. Further, it depends on the present weed species and the management at the field site (Bàrberi, 2002). The weather conditions at both field sites, with sufficient amount and distribution of rainfall and the long growing period, were very suitable to achieve a dense canopy and competitive plant stand to suppress weeds. The biomass production of CCs, does not necessarily need to correlate with their weed suppression ability (Finney et al., 2016; Kunz et al., 2016). However, biomass-driven CCs are generally more competitive (Finney et al., 2016; Teasdale, 1996). Instead of single CCs species, a species mixture was used within this study. By combining different CC species with specific advantages in CC mixtures, the benefits concerning weed, soil, nutrient, and pest management may increase (Bàrberi and Mazzoncini, 2001; Malézieux et al., 2009).

The continuous loss of herbicides in the EU (Melander et al., 2013) and the increasing problems with herbicide resistant weeds will raise the awareness of producers to strengthen their focus on non-chemical weed management. The GLY+GLY treatment achieved the significantly highest WCE within this experiment. However, a single glyphosate application (GLY) was not sufficient, in particular, to control *A. myosuroides* weeds. *A. myosuroides* plants emerge in several flushes during autumn, when the climate conditions are favorable (Colbach et al., 2006). Applying glyphosate too early might miss most of the plants. Furthermore, this study demonstrated that at both trials, during autumn and at the end of the growing period, CCs (especially CC+MT) had similar effects on WCE, ACE, and VCE as the chemical treatments. The CC+NT treatment was only showing a slightly weaker WCE and VCE than the CC+MT treatment. Nevertheless, glyphosate is a useful tool within no-till and reduced tillage systems.

The wheat residue management (presence or absence of straw) had a minor effect on the success of either mechanical, chemical, or biological weed control practices. Even though burning the straw on the field is used in some regions and it might result in decreasing weed numbers, it can have some negative side effects especially concerning the carbon gas emissions. The baling of straw is not achieving a decrease of the weed infestation (Moss, 1979), which was also demonstrated within this study.

Within this study, the effects of postharvest weed control on the previous spring crop season were not evaluated, but might deliver interesting insights to see whether the CC treatments preserved weed seeds, instead of reducing the weed seed bank for the repeated stubble tillage treatments (FSL, DST).

2.2.6. CONCLUSIONS

The flat soil tillage with rotary harrow (FST) and the deep soil tillage with wing share cultivation (DSL) treatments did not show satisfying results concerning WCE and ACE, compared to the chemical and biological methods, but seemed to be a suitable tool for volunteer wheat control. The cover crop (CC) suppression performance for total weed and especially for *A. myosuroides* showed, that even conservation practices have the potential to minimize future weed control challenges. Their success mainly attributes to their fast and competitive development, which is determined by external factors. In a season with unfavorable growing conditions for CCs, stubble tillage and glyphosate applications might be more efficient weed control practices. Even though the weed suppression ability of CCs is often unpredictable, it is worthwhile to do cover-cropping in terms of soil conservation and biodiversity. The effect of non-chemical weed management in reduced and no-till systems still needs a better understanding for weed dynamics (Melander et al., 2013). Long-term experiments will help to show how continuous stubble tillage, herbicide application and cover-cropping will affect the weed density and the weed community and which combinations will enable a sufficient and sustainable weed control.

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2.2.7. APPENDIX A

Table 2.2.4-4: Mean number of weed species (averaged over all counting dates) per m^2 for the eight treatments (averaged for the treatments with the presence and the absence of straw) at the field sites Binsen and Risp. 1: untreated control; 2: flat soil tillage; 3: deep soil tillage; 4: ploughing; 5: single glyphosate application; 6: dual glyphosate application; 7: cover crop mixture + mulch-till; 8: cover crop mixture + no-till. Alopecurus myosuroides Huds. (A. myosuroides), Veronica persica Poir. (V. Persica), Thalaspi arvense L. (T. arvense), Lamium purpureum L. (L. purpureum), Stellaria media Vill. (S. media), Raphanus raphanistrum L. (R. raphanistrum). Others: Cirsium arvense (L.) Scop., Sonchus arvensis L., Matricaria chamomilla L., Euphorbia helioscopia L., Borago officinalis L..

	Binsen								**Risp**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A. myosuroides	7	21	19	15	13	6	2	1	9	14	14	9	14	5	1	2
Volunteer wheat	73	8	6	6	4	4	1	2	139	16	18	11	10	9	3	14
V. persica	6	4	2	7	-	-	6	3	12	5	7	14	2	-	11	10
T. arvense	2	-	-	14	-	1	-	-	5	-	-	11	-	-	-	-
L. purpureum	3	4	2	5	-	-	4	4	5	4	6	13	1	1	7	12
S. meida	2	-	1	8	1	1	-	-	4	-	-	12	-	-	1	1
R. raphanistrum	1	1	1	3	-	-	-	-	2	-	1	3	-	-	-	-
Others	3	1	-	2	-	-	-	-	7	-	-	5	-	-	-	-

2.2.8. REFERENCES

BARBERI P (2002) Weed management in organic agriculture: are we addressing the right issues? *Weed research* **42,** 177–193.

BÀRBERI P & MAZZONCINI M (2001) Changes in weed community composition as influenced by cover crop and management system in continuous corn. *Weed Science* **49,** 491–499.

BILALIS D, SIDIRAS N, ECONOMOU G & VAKALI C (2003) Effect of Different Levels of Wheat Straw Soil Surface Coverage on Weed Flora in Vicia faba Crops. *Journal of Agronomy and Crop Science* **189,** 233–241.

BLUBAUGH CK, HAGLER JR, MACHTLEY SA & KAPLAN I (2016) Cover crops increase foraging activity of omnivorous predators in seed patches and facilitate weed biological control. *Agriculture, Ecosystems & Environment* **231,** 264–270.

BOSTRÖM U (1999) Type and time of autumn tillage with and without herbicides at reduced rates in southern Sweden. *Soil and Tillage Research* **50,** 271–281.

BRUST J, CLAUPEIN W & GERHARDS R (2014) Growth and weed suppression ability of common and new cover crops in Germany. *Crop Protection* **63,** 1–8.

BRUST J, GERHARDS R, KARANISA T, RUFF L & KIPP A (2011) Why undersown and cover crops become important again for weed suppression in European cropping systems. *Gesunde Pflanzen* **63,** 191–198.

CIRUJEDA A & TABERNER A (2006) Relating Weed Size, Crop Soil Cover and Soil Moisture with Weed Harrowing Efficacy on Papaver rhoeas and Other Dicotyledoneous Weeds in Mediterranean Conditions. *Biological Agriculture & Horticulture* **24,** 181–195.

CLEMENTS RO, MARTYN TM, DONALDSON G & WOLFE M (2000) Clover: cereal bi-cropping for organic farms. *Aspects of Applied Biology,* 181–185.

COLBACH N, DÜRR C, ROGER-ESTRADE J, CHAUVEL B & CANEILL J (2006) AlomySys: Modelling black-grass (Alopecurus myosuroides Huds.) germination and emergence, in interaction with seed characteristics, tillage and soil climate. *European Journal of Agronomy* **24,** 95–112.

DAO TH (1991) Field Decay of Wheat Straw and its Effects on Metribuzin and S-Ethyl Metribuzin Sorption and Elution from Crop Residues. *Journal of Environmental Quality* **20,** 203–208.

DAVIES LR & NEVE P (2017) Interpopulation variability and adaptive potential for reduced glyphosate sensitivity in Alopecurus myosuroides. *Weed research* **57,** 323–332.

DEUTSCHE SAATVEREDELUNG AG TerraLife - Vita Maxx TR. https://www.dsv-saaten.de/zwischenfruechte/terralife/mischungen/vitamaxx-tr.html, last accessed 14 Sep 2018.

DEVITA P, DIPAOLO E, FECONDO G, DIFONZO N & PISANTE M (2007) No-tillage and conventional tillage effects on durum wheat yield, grain quality and soil moisture content in southern Italy. *Soil and Tillage Research* **92,** 69–78.

FINNEY DM, WHITE CM & KAYE JP (2016) Biomass Production and Carbon/Nitrogen Ratio Influence Ecosystem Services from Cover Crop Mixtures. *Agronomy Journal* **108,** 39–52.

FROUD-WILLIAMS RJ, CHANCELLOR RJ & DRENNAN DSH (1984) The Effects of Seed Burial and Soil Disturbance on Emergence and Survival of Arable Weeds in Relation to Minimal Cultivation. *The Journal of Applied Ecology* **21,** 629.

GRUBER S & CLAUPEIN W (2009) Effect of tillage intensity on weed infestation in organic farming. *Soil and Tillage Research* **105,** 104–111.

GRUBER S, PEKRUN C, MÖHRING J & CLAUPEIN W (2012) Long-term yield and weed response to conservation and stubble tillage in SW Germany. *Soil and Tillage Research* **121,** 49–56.

HARTWIG NL & AMMON HU (2002) Cover crops and living mulches. *Weed Science* **50,** 688–699.

HATCHER PE & FROUD-WILLIAMS RJ, eds (2017) Weed research: Expanding horizons. Non-chemical weed management. John Wiley & Sons Ltd, Hoboken, NJ.

HEAP I (2018) The international survey of herbicide resitant weeds. www.weedscience.org, last accessed 7 Sep 2018.

HERNANZ J, LÓPEZ R, NAVARRETE L & SÁNCHEZ-GIRÓN V (2002) Long-term effects of tillage systems and rotations on soil structural stability and organic carbon stratification in semiarid central Spain. *Soil and Tillage Research* **66,** 129–141.

KUNZ C, STURM DJ, VARNHOLT D, WALKER F & GERHARDS R (2016) Allelopathic effects and weed suppressive ability of cover crops.

LANGDALE GW, BLEVINS RL, KARLEN DL et al. (1991) Cover crop effects on soil erosion by wind and water. *Cover crops for clean water,* 15–22.

LICHTFOUSE E, ed (2009) Sustainable agriculture. Springer, Dordrecht.

MACHLEB J, KOLLENDA BL, PETEINATOS GG & GERHARDS R (2018) Adjustment of Weed Hoeing to Narrowly Spaced Cereals. *Agriculture* **8,** 54.

MALÉZIEUX E, CROZAT Y, DUPRAZ C et al. (2009) Mixing Plant Species in Cropping Systems: Concepts, Tools and Models: A Review. In: *Sustainable agriculture* (ed. E LICHTFOUSE), 329–353. Springer, Dordrecht.

MANTLE PG & SHAW S (1976) Role of ascospore production by Claviceps purpurea in aetiology of ergot disease in male sterile wheat. *Transactions of the British Mycological Society* **67,** 17–22.

MELANDER B, HOLST N, RASMUSSEN IA & HANSEN PK (2012) Direct control of perennial weeds between crops – Implications for organic farming. *Crop Protection* **40,** 36–42.

MOSS S (2017) Black-grass (Alopecurus myosuroides): Why has this Weed become such a Problem in Western Europe and what are the Solutions? *Outlooks on Pest Management* **28,** 207–212.

MOSS SR (1979) The influence of tillage and method of straw disposal on the survival and growth of black-grass, Alopecurus myosuroides, and its control by chlortoluron and isoproturon. *Annals of Applied Biology* **91,** 91–100.

NORRIS RF & KOGAN M (2000) Interactions between weeds, arthropod pests, and their natural enemies in managed ecosystems. *Weed Science* **48,** 94–158.

PEKRUN C & CLAUPEIN W (2006) The implication of stubble tillage for weed population dynamics in organic farming. *Weed research* **46,** 414–423.

PETIT S, TRICHARD A, BIJU-DUVAL L, MCLAUGHLIN Ó & BOHAN DA (2017) Interactions between conservation agricultural practice and landscape composition promote weed seed predation by invertebrates. *Agriculture, Ecosystems & Environment* **240,** 45–53.

POWLES SB & YU Q (2010) Evolution in action: plants resistant to herbicides. *Annual review of plant biology* **61,** 317–347.

RASMUSSEN J (1991) A model for prediction of yield response in weed harrowing. *Weed research* **31,** 401–408.

S. S. SNAPP, S. M. SWINTON, R. LABARTA et al. (2005) Evaluating Cover Crops for Benefits, Costs and Performance within Cropping System Niches. *Agronomy Journal* **97,** 322–332.

SCHAPPERT A, MESSELHÄUSER M, SAILE M, PETEINATOS G & GERHARDS R (2018) Weed Suppressive Ability of Cover Crop Mixtures Compared to Repeated Stubble Tillage and Glyphosate Treatments. *Agriculture* **8,** 144.

TEASDALE JR (1996) Contribution of Cover Crops to Weed Management in Sustainable Agricultural Systems. *Journal of Production Agriculture* **9,** 475–479.

WESTERMAN P, HOFMAN A, VET L & VAN DER WERF W (2003) Relative importance of vertebrates and invertebrates in epigeaic weed seed predation in organic cereal fields. *Agriculture, Ecosystems & Environment* **95,** 417–425.

WORTMAN SE, FRANCIS CA, BERNARDS ML, DRIJBER RA & LINDQUIST JL (2012) Optimizing Cover Crop Benefits with Diverse Mixtures and an Alternative Termination Method. *Agronomy Journal* **104,** 1425–1435.

WRUCKE MA & ARNOLD WE (1985) Weed Species Distribution as Influenced by Tillage and Herbicides. *Weed Science* **33,** 853–856.

2.3. Effect of cinmethylin against *Alopecurus myosuroides Huds.* in winter cereals

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2.3.1. ABSTRACT

Cinmethylin is a potential new selective pre-emergence herbicide in inhibiting the fatty acid thioesterases (FAT). It is effective against *Alopecurus myosuroides* Huds. and other grass-weeds in winter cereals and oil-seed rape. Five field experiments were conducted in Southwestern Germany from 2018 until 2020 to assess the control efficacy of cinmethylin and other common pre – emergence herbicides and combinations of herbicides against *A. myosuroides* and the yield response of winter wheat and winter triticale. In four experiments, the effect of early and late sowing of winter cereals was included as second factor in the experiment to investigate if late sowing can reduce *A. myosuroides* density and increase weed control efficacy. All fields were heavily infested with *A. myosuroides* with average densities of 105-730 plants/m^2 in the early sown controls. Late sowing reduced weed densities in three out of four experiments. Herbicides controlled 42 – 100% of the *A. myosuroides* plants. However, none of the treatments was consistently better than the other treatments. In three out of five experiments, grain yields were significantly increased by the herbicide treatments. The results demonstrate that cinmethylin is a new option for controlling *A. myosuroides* in winter cereals. However, it needs to be combined with other weed control tactics.

Keywords: black-grass; ALS inhibitors; ACCase inhibitors; very long chained fatty acid inhibitors; IWM; seeding time

2.3.2. INTRODUCTION

Alopecurus myosuroides Huds. (black-grass) is a problematic weed species in Western European winter cereal production. Densities increased within the last four decades due to rotations with high proportions of winter cereals, reduced tillage and early sowing dates in September and early October, when most of the seeds germinate (Lutman *et al.*, 2013; Moss, 2017b; Melander, 1995; Moss, 1990). *A. myosuroides* prefers fertile and moist soils with high organic matter and clay contents (Lutman *et al.*, 2013). At those locations, it is very competitive and causes yield losses in winter wheat of 15-20% at densities of 100 plants/m^2 (Blair *et al.*, 1999; Lutman *et al.*, 2013; Gerhards *et al.*, 2016). In England, France, Germany, Belgium and the Netherlands, *A. myosuroides* populations have been identified as resistant to standard herbicide applications (Heap, 2014a; Délye *et al.*, 2007; Drobny *et al.*, 2006; Neve, 2007). Populations with evolved resistance to herbicides in Europe have been documented resistance mainly against the post-emergence herbicide groups of acetyl CoA carboxylase (ACCase)-inhibitors and acetolactate synthase (ALS)-inhibitors (Heap, 2014a; Menne & Hogrefe, 2012). Pre-emergence herbicides are less affected by resistance (Bailly *et al.*, 2012). However, their weed control efficacy is usually lower than for post-emergence herbicides and sufficient soil moisture is necessary for root uptake. Prosulfocarb, flufenacet, pendimethalin, and diflufenican are the most common pre-emergence herbicides used in European winter cereal production (Bailly *et al.*, 2012). Few studies have confirmed resistance to flufenacet in several populations of *A. myosuroides* (Dücker *et al.*, 2019; Menne & Hogrefe, 2012). Resistance mechanism is based on enhanced metabolism induced by increased Glutathion-S-transferase activity. Cinmethylin is a potential new herbicide against *A. myosuroides* and other grass-weeds. Originally the benzylether cinmethylin was developed by the

Shell Chemical Company as a herbicide against grass-weeds in rice (Dayan, 2019). Campe et al. (2018) explored the mode of action of cinmethylin. It inhibits the fatty acid thioesterases (FAT) in the plastids. Similar to other pre-emergence herbicides, it is taken up predominantly by the roots of the plants. It controls grass-weeds such as *A. myosuroides*, *Apera spica-venti (L.) Beauv*. and *Lolium spp*. *(Ryegrass)* in winter cereals and oil-seed rape. Since cinmethylin has not yet been used in Europe it is assumed that *A. myosuroides* populations are still sensitive to this herbicide since selection for resistance never happened. The concept of Integrated Weed Management (IWM) is to combine multiple tactics of weed control (Harker, 2013). This can slow down selection for herbicide resistant populations. In this study, pre-emergence cinmethylin was combined with alternate sowing dates for winter wheat. *A. myosuroides* is well adapted to early sowing dates in September (Moss & Clarke, 1994; Lutman *et al.*, 2013). Late sowing of winter wheat in October and November significantly reduced *A. myosuroides* emergence (Menegat & Nilsson, 2019; Gerhards *et al.*, 2016) and still provided sufficient time for vegetative wheat development.

The objective of this study was to test the efficacy against *A. myosuroides* and crop response of the new pre-emergence herbicide cinmethylin at different locations over three years in winter wheat compared to other pre- and post-emergence herbicides. Our first hypothesis was that (i) cinmethylin efficacy against *A. myosuroides* was higher than for other commonly used flufenacet based pre-emergence herbicides. The second hypothesis was that (ii) late sowing of winter cereals in the end of October and November in combination with a cinmethylin application reduces *A. myosuroides* infestation rates compared to early sowing dates in combination with a cinmethylin application.

2.3.3. MATERIAL AND METHODS

2.3.3.1. Experimental sites

Five field experiments were conducted in winter wheat and winter triticale in the Southwestern Germany from 2017 until 2020. Experiments were located at the research station Ihinger Hof (48.44°N, 8.55°E) of the University of Hohenheim, in Entringen 48.33°N,8.57°E) and in Hirrlingen (48.25°N, 8.52°E). Climatic conditions are similar at all locations. Average monthly temperatures and precipitation in comparison to the long-term means from 2017 until 2020 are shown in Table 2.3.3-1. The average temperatures were higher than the long-term average in all three years. The autumns in 2018 and 2019 were extremely dry. Annual average temperature was exceeded. The soil properties (parabrown) of the locations were quite similar with the first 300 mm of soil consisting of 4% sand, 40% clay and 56% silt. Experimental details of the crops, cultivars, sowing dates and seed density are given in Table 2.3.3-2.

Table 2.3.3-1: Average monthly temperatures and precipitation at Ihinger Hof Research Station from October 2017 until August 2020 and long-term means from 1961 until 1990.*

	Mean temperatures (°C)					Mean precipitation (mm)				
	2017	2018	2019	2020	Long term average	2017	2018	2019	2020	Long term average
Jan		3.9	-0.9	2.3	-0.4		89.0	45.6	11.2	50.0
Feb		-2.4	3.5	4.8	0.7		19.4	13.1	88.2	45.0
March		2.9	6.1	4.7	4.0		21.2	47.1	49.7	51.3
April		12.4	8.6	10.9	7.9		17.4	26.7	4.8	60.1
May		14.9	10.1	11.9	12.2		75.1	107.2	45.6	80.1
June		17.4	18.5	15.5	15.5		32.5	52.2	85.4	92.6
July		19.9	18.7	18.3	17.5		32.0	53.9	15.3	67.5
Aug		19.6	18.2	19.3	16.8		28.8	82.4	11.2	73.6
Sep		14.8	13.7		13.6	5.8	78.0	28.4		57.2
Oct	10.3	10.1	10.8		9.0	51.1	26.4	53.6		45.2
Nov	4.0	4.5	3.9		3.7	63.0	19.5	43.4		62.0
Dec	1.1	2.6	2.7		0.7	32.5	83.9	37.4		53.3

**source (“wetter-bw”, 2020)*

Table 2.3.3-2: Experimental details of the field experiments.

No. of experiment	Location	Crop (cultivar)	Year	Sowing date	Seeding rate (seeds/m²)
1	Ihinger Hof	winter-wheat (RGT-Reform)	2018	19.10.2017 04.12.2017	300 350
2	Ihinger Hof	winter-triticale (Tulus)	2019	25.09.2018 25.10.2018	250 350
3	Entringen	winter-triticale (Tulus)	2019	08.10.2018 10.11.2018	250 350
4	Hirrlingen	winter-wheat (RGT-Reform)	2019	06.10.2018	400
5	Ihinger Hof	winter-wheat (Patras)	2020	08.10.2019 31.10.2019	250 380

2.3.3.2. Experimental design

The experiments at Ihinger Hof and Entringen were realized as a two factorial randomized complete block design with three repetitions. The first factor was the weed control method including 6 herbicide treatments and an untreated control. The second factor was the sowing time of winter cereals including an early and late date. The experiment at Hirrlingen was a randomized complete block design with three replications. It contained one factor including 12 herbicide treatments and an untreated control. The plot size in all experiments was 3 m x 12 m. The herbicides tested and times of application are presented in Table 2.3.3-3. Herbicides were applied with a plot sprayer (Schachtner-Gerätetechnik, Ludwigsburg, Germany), which was calibrated for a volume of 200 L/ha. The application dates were adapted to the individual years. Pre-emergence herbicides were applied 5 days after sowing (DAS) in BBCH 10 – 11 of the crop. Post-emergence herbicides were sprayed in BBCH 10 – 13 (PF) respectively 21 – 29 of the crop. Seedbed quality differed between the years according to the weather conditions in autumn. In 2018 seedbed was extremely rough with usable field capacity < 30% at the time of herbicide application, afterwards soil moisture was increased by sufficient rain falls. Broadleaved weed species were controlled in all plots with synthetic auxins in spring. Other pesticides and fertilizers were applied according to good professional practice.

Table 2.3.3-3: Herbicide treatments tested in the five field experiments.

Treatment	**Active ingredients**	**Locations**	**Application rate**	**Years**	**Crops**	**Time of application (DAS =days after sowing)**
Atlantis® WG (IM)	*29.2 g/kg mesosulfuron, 5.6 g/kg iodosulfuron*	Ihinger Hof,	500 g/ha	2018	winter wheat	140
Atlantis Flex (IM)	*45 g/kg mesosulfuron-methyl, 67.5 g/kg proboxycarbazone*	Ihinger Hof, Entringen, Hirrlingen	0.3 L/ha	2019, 2020	winter wheat winter triticale	140
Avoxa (PP)	*33.3 g/ Lpinoxaden, 8.3 g/L pyroxsulam, 8.3 g/L cloquintocet-mexyl*	Hirrlingen	1.8 L/ha	2019	winter wheat	140
Axial 50 (PIN)	*50 g/L pinoxaden, 12.5 g/L cloquintocet-mexyl*	Hirrlingen	0.9 L/ha	2019	winter wheat	140
Broadway	*68.3 g/kg pyroxsulam, 22.8*	Hirrlingen	220 g/ha	2019	winter	140

(PFC)	*g/kg florasulam, 68.3 g/kg cloquintocet-mexyl*				wheat	
Malibu (PF)	*60 g/L flufenacet, 300 g/L pendimethalin*	Hirrlingen	4 L/ha	2019	winter wheat	25
Herold SC (FD)	*400 g/L flufenacet, 200 g/L diflufenican*	Hirrlingen	0.6 L/ha	2019	winter wheat	5
Boxer (PRO)	*800 g/L prosulfocarb*	Hirrlingen	5 L/ha	2019	winter wheat	5
Cadou SC (FLU)	*508.8 g/L flufenacet*	Ihinger Hof, Entringen, Hirrlingen	0.5 L/ha	2018, 2019, 2020	winter wheat, winter triticale	5
Pontos (FP)	*240 g/L flufenacet, 100 g/L picolinafen*	Ihinger Hof, Entringen, Hirrlingen	1 L/ha	2018, 2019, 2020	winter wheat, winter	5

					triticale	
Luximo (reduced) (CIN 375)	*750 g/L cinmethylin*	Hirrlingen	0.5 L/ha	2019	winter wheat	5
Luximo (full) (CIN 495) (CIN)	*750 g/L cinmethylin*	Ihinger Hof, Entringen, Hirrlingen	0.66 L/ha	2018, 2019, 2020	winter wheat, winter triticale	5
Luximo and Pontos (CFP)	*750 g/L cinmethylin, 240 g/L flufenacet, 100 g/L picolinafen*	Ihinger Hof, Entringen, Hirrlingen	0.5 L/ha 0.45 L/ha	2018, 2019, 2020	winter wheat, winter triticale	5

2.3.3.3. Assessments

Density of *A. myosuroides* was determined 45, 120 and 180 DAS. In the figures, data of the second counting of *A. myosuroides* are presented. Density of cereal plants was determined recorded 28 days after herbicide application. Plants were counted in a 0.1 m^2 frame placed four times in each plot. Grain yield was measured in a 1.5 m x 12 m strip in each plot with a plot harvester (Wintersteiger, Elite 3, Ried im Innkreis, Austria). Grain yields were transformed to a homogenous moisture of 14%.

2.3.3.4. Statistical analysis

For data analysis, the statistical software R (Version 3.6.2, RStudio Team, Boston, MA, USA) was used. Prior to ANOVA, the data were checked for homogeneity of variance and normal distribution of residuals. If necessary, data were square root transformed to homogenize variances and to normalize the distribution. In the figures, back transformed means are shown. In the ANOVA, herbicide treatment, date of sowing and the interactions were included as fixed effects. Multiple mean comparison tests were performed using the Tukey HSD-Test at a significance level of $\alpha \leq 0.05$.

2.3.4. RESULTS

2.3.4.1. Herbicide test at Hirrlingen

In the winter wheat experiment at Hirrlingen 2019, the highest density of 122 plants/m^2 was recorded in the CON, followed by the post-emergence combination of IM with 69 plants/m^2 and PIN with 63 plants/m^2. The lowest density of four *A. myosuroides* plants/m^2 was counted in the treatment within the recommended field rate of cinmethylin (CIN 495) followed by the combination of CFP with 7 plants/m^2 (Figure 2.3.4-1a). Highest *A. myosuroides* control efficacy was achieved by the treatments with CIN 495 (98%) and CFP (94%), compared to the CON. The treatments of IM and PIN had the lowest control efficacies of 43% and 45%. *A. myosuroides* control efficacy of the other treatments ranged between 58-82% (Figure 2.3.4-1b). Highest percentages of emerged winter wheat plants were achieved by the treatments of FLU and FP with 80% and 85%. The lowest percentage of 61% was recorded in the treatment of FD (Figure 2.3.4-1c). Grain yield of winter wheat was lowest in the CON with 5.7 t/ha. Highest grain yields were recorded in the treatments of CFP (12.4 t/ha), FP (11.9 t/ha) and CIN 495 (11.7 t/ha) (Figure 2.3.4-1d).

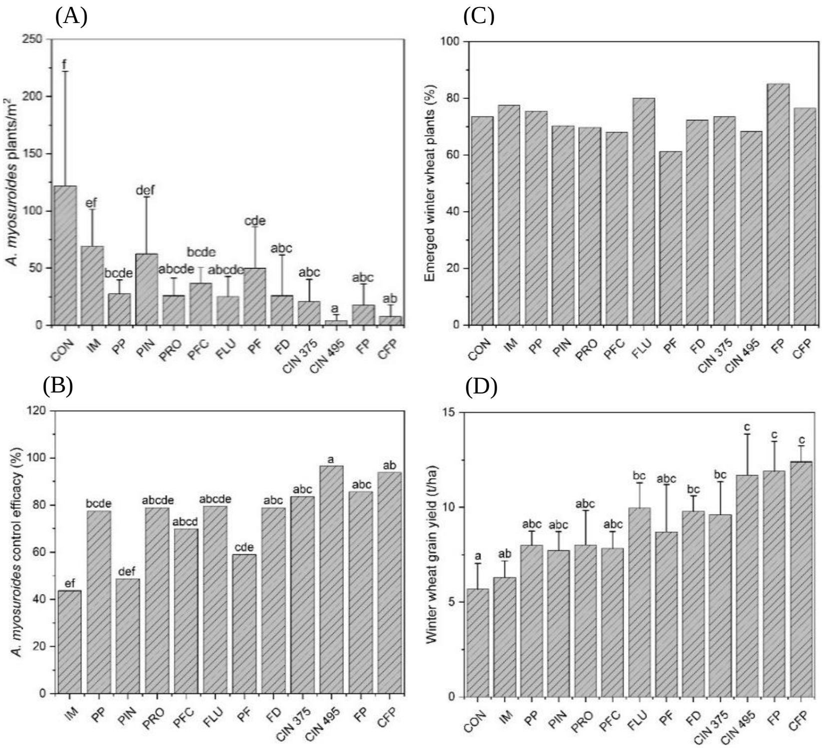

Figure 2.3.4-1: A. myosuroides density/m² (a), A. myosuroides control efficacy (%) (b), emerged winter wheat plants (%) according to seeding rate (c) and winter wheat grain yield (t/ha) (d) recorded at Hirrlingen 2019. Means with the same letter are not significantly different according to Tukey HSD test at α ≤ 0.05. CON = untreated control, IM = iodosulfuron + mesosulfuron, PP = pinoxaden + pyroxsulam, PIN = pinoxaden, PRO = prosulfocarb, PFC = pyroxsulam + florasulam, FLU = flufenacet, PF = flufenacet + pendimethalin, FD = flufenacet + diflufenican, CIN 375 = 375 g a. i. cinmethylin, CIN 495 = 495 g a.i. cinmethylin, FP = flufenacet + picolinafen, CFP = cinmethylin + flufenacet + picolinafen.

2.3.4.2. *A. myosuroides* density at the experimental sites Ihinger Hof and Entringen

In all four experiments, infestations rates with *A. myosuroides* were high with densities of more than 100 plants/m^2 in the early sown control plots. Late seeding of winter cereals reduced *A. myosuroides* densities except for the 2018 experiment at Ihinger Hof (Figure 2.3.4-2b). Pre-emergence herbicides and combinations with post-emergence herbicides significantly reduced *A. myosuroides* densities compared to the untreated control except for the early seeding plots of FLU and FIM at Ihinger Hof in 2017 (Figure 2.3.4-2a) and the late seeding treatments of FLU at Ihinger Hof in 2019 (Figure 2.3.4-2c). None of the herbicide treatments provided better weed control efficacies than all other treatments consistently over all experiments.

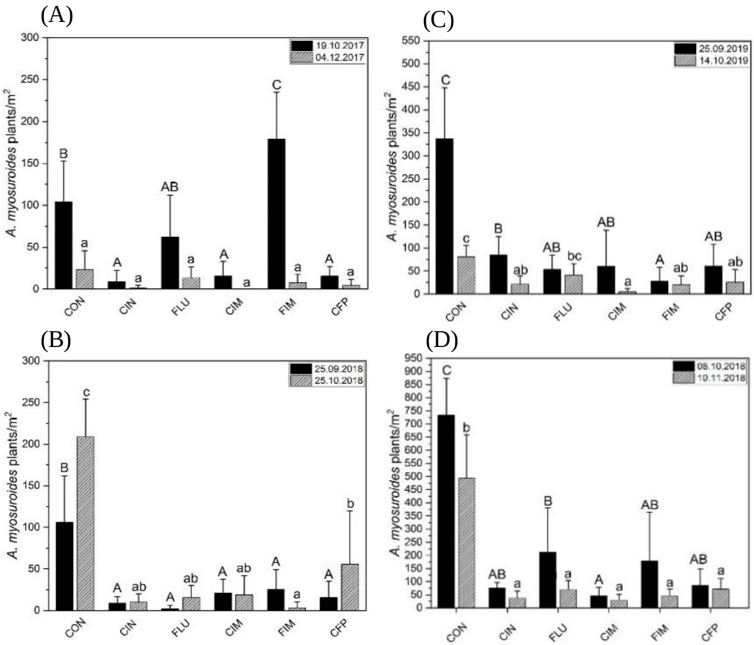

Figure 2.3.4-2: A. myosuroides density/m² recorded at Ihinger Hof (a, b, c) and Entringen (d) in April 2018 – 2020. Black bars represent the early seeding date and grey bars the late seeding date. The date of seeding per year is included in the upper right corner. Means with different capital letters show significant differences within the early seeding date according to Tukey HSD test at $\alpha \leq 0.05$. Means with different small letters show significant differences within the late seeding date according to Tukey HSD test at $\alpha \leq 0.05$. CON = untreated control, CIN = cinmethylin, FLU = flufenacet, CIM = cinmethylin + iodosulfuron + mesosulfuron, FIM = flufenacet + iodosulfuron + mesosulfuron, CFP = cinmethylin + flufenacet + picolinafen.

2.3.4.3. *A. myosuroides* control efficacy at Ihinger Hof and Entringen

In three out of the four experiments (Figure 2.3.4-3), the treatments with CIN, CIM and CFP achieved control efficacies above 90%. Late seeding of winter cereals resulted in higher *A. myosuroides* control efficacies at Ihinger Hof in all years compared to the early seeding date, except for the treatment of CFP. At Entringen however, *A. myosuroides* control efficacies were higher in the early sown plots.

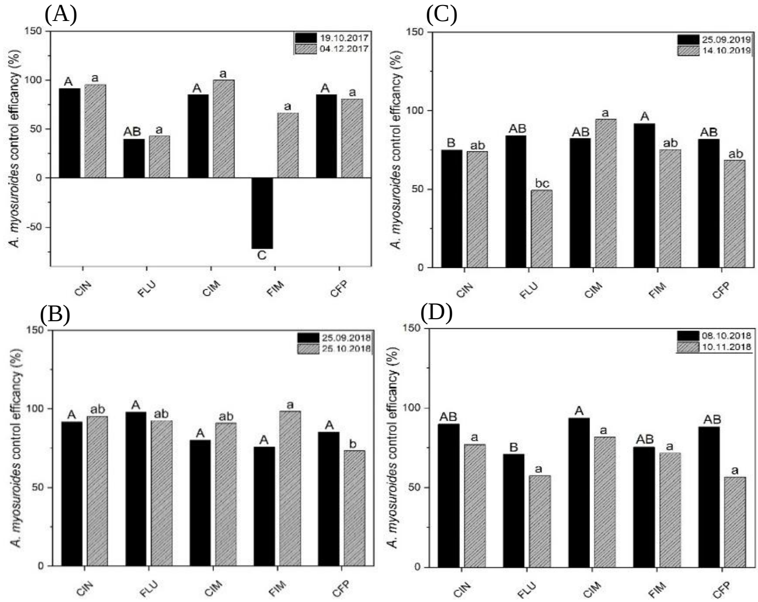

Figure 2.3.4-3: A. myosuroides control efficacy (%) recorded at Ihinger Hof (a, b, c) and Entringen (d) in April 2018 – 2020. Black bars show the early seeding date, while grey bars the late seeding date. The date of seeding per year is included in the upper right corner. Means with different capital letters show significant differences within the early seeding date according to Tukey HSD test at α ≤ 0.05. Means with different small letters show significant differences within the late seeding date according to Tukey HSD test at α ≤ 0.05. CON = untreated control, CIN = cinmethylin, FLU = flufenacet, CIM = cinmethylin + iodosulfuron + mesosulfuron, FIM = flufenacet + iodosulfuron + mesosulfuron, CFP = cinmethylin + flufenacet + picolinafen.

2.3.4.4. Emerged cereal plants at the experimental sites Ihinger Hof and Entringen

Percentages of emerged cereal plants were mostly higher with above 60% in the early sown treatments at Ihinger Hof in all three years, except for the CON in 2018 with 57%. At the experimental site of Entringen emerged cereal plants ranged between 25.8% - 22.8% for the early sown treatments and 13.7% - 21.9% for the late sown treatments (Table 2.3.4-1). None of the herbicide treatments consistently reduced cereal plants.

Table 2.3.4-1: Winter cereal density (%) from 2018-2020 of four experiments. Percentages of emerged cereal plants were calculated according to the corresponding seeding rate. Means with the same letter are not significantly different according to Tukey HSD-test at $p \leq 0.05$. Levels of significance are shown behind the result for each experiment separately. Results without letters were no significant differences were detected according to ANOVA. CON = untreated control, CIN = cinmethylin, FLU = flufenacet, CIM = cinmethylin + iodosulfuron + mesosulfuron, FIM = flufenacet + iodosulfuron + mesosulfuron, CFP = cinmethylin + flufenacet + picolinafen.

Treatment	Sowing date	Ihinger Hof 2018	Ihinger Hof 2019	Entringen 2019	Ihinger Hof 2020
		Emerged cereal plants (%)			
CON		56.7^{a}	66.0^{a}	32.0^{a}	64.8^{a}
CIN	19.10.2017	70.7^{ab}	86.0^{a}	33.8^{a}	69.6^{a}
FLU	25.09.2018	60.3^{ab}	70.0^{a}	28.0^{a}	61.6^{a}
CIM	08.10.2018	67.3^{ab}	85.6^{a}	25.8^{a}	72.0^{a}
FIM	08.10.2019	75.7^{ab}	98.0^{a}	29.8^{a}	64.8^{a}
CFP		77.7^{b}	83.2^{a}	29.8^{a}	80.8^{a}
CON		43.7^{B}	38.6^{A}	21.1^{A}	40.6^{A}
CIN	04.12.2017	14.6^{A}	56.6^{A}	13.7^{A}	57.7^{A}
FLU	25.10.2018	42.9^{B}	57.4^{A}	21.9^{A}	83.1^{A}
CIM	10.11.2018	8.6^{A}	58.0^{A}	17.6^{A}	57.4^{A}
FIM	31.10.2019	58.0^{C}	55.7^{A}	17.6^{A}	59.1^{A}
CFP		35.1^{B}	55.4^{A}	17.6^{A}	58.9^{A}

2.3.4.5. Grain yield of winter wheat and triticale at Ihinger Hof and Entringen

Grain yield of winter cereals were mostly higher in the early sown treatments except for the CON at Ihinger Hof 2018, FIM at Ihinger Hof 2020 and CON and CIM in Entringen in 2019. Herbicide treatments significantly increased grain yield in two experiments (Ihinger Hof 2020 and Entringen 2019). None of the herbicide treatments consistently resulted in higher yields than all other herbicide treatments (Figure 2.3.4-4).

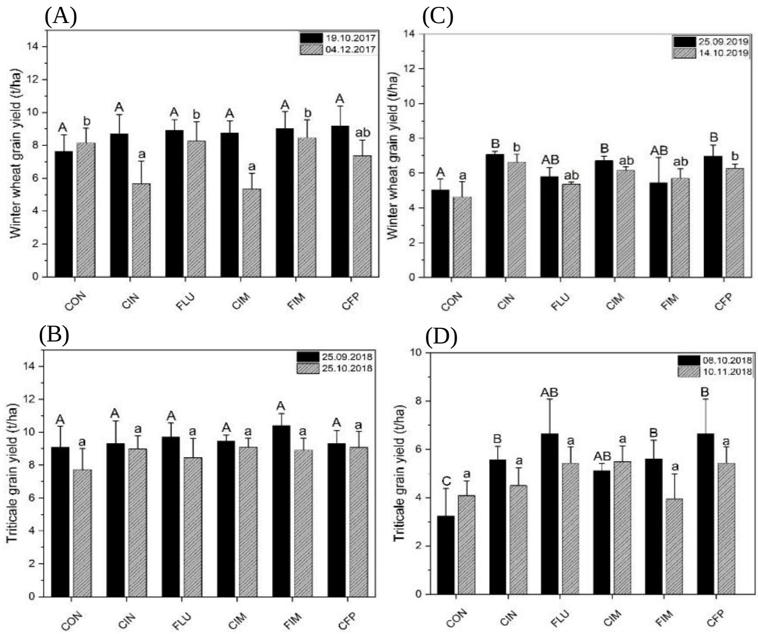

Figure 2.3.4-4: Winter wheat grain yield (t/ha) recorded at Ihinger Hof (a, b, c) and Entringen (d) in 2018 – 2020. Black bars show the early seeding date, while grey bars the late seeding date. The date of seeding per year is included in the upper right corner. Means with different capital letters show significant differences within the early seeding date according to Tukey HSD test at α ≤ 0.05. Means with different small letters show significant differences within the late seeding date according to Tukey HSD test at α ≤ 0.05. CON = untreated control, CIN = cinmethylin, FLU = flufenacet, CIM = cinmethylin + iodosulfuron + mesosulfuron, FIM = flufenacet + iodosulfuron + mesosulfuron, CFP = cinmethylin + flufenacet + picolinafen.

2.3.5. DISCUSSION

Cinmethylin controlled more than 90% of *A. myosuroides* plants when averaged over the five field experiments. Average control efficacy for flufenacet was 75%. However, cinmethylin efficacy was not consistently higher than the other pre-emergence herbicides. Therefore, the first hypothesis needs to be rejected. Control efficacies of pre-mergence herbicides against *A. myosuroides* were similar to other studies of Bailly *et al.* (2012) such as flufenacet with 98% and 95% – 97% for the combination of flufenacet and pendimethalin and 90% – 98% for flufenacet and diflufenican. Menne *et al.* (2012b) observed slightly higher weed control efficacies with the combination of flufenacet and diflufenican of 70% – 95%. Variations in soil water content, limited soil persistence and a long period of emergence of *A. myosuroides* from autumn until spring can explain the difference in weed control efficacy of pre-emergence herbicides (Kudsk & Kristensen, 1992). Furthermore, pre-emergence herbicides can be lost due to surface evaporation or leaching in wet soils (Kudsk & Kristensen, 1992; Hammerton, 1967). Due to limited selectivity, pre-emergence of such herbicides also cause a risk of crop damage when they are applied under unfavorable conditions such as low temperatures (Robinson *et al.*, 2015). In the 2017 experiment, cinmethylin was applied in January in winter wheat. This treatment caused a significant grain yield loss for the late seeding date was recorded.

The second hypothesis was proofed in the current studies that late sowing of winter cereals in autumn reduced densities of *A. myosuroides* by approximately 50% in three out of four experiments. In combination with cinmethylin, late sowing even achieved higher control levels of above 75%. Similar reduced emergence of *A. myosuroides* with late seeding was observed by Lutman *et al.* (2013), Gerhards et al. (2016), Menegat & Nilsson

(2019). Seeds of *A. myosuroides* have the highest germination rate in September in Western European growing conditions (Moss, 2017b).

The results of the five field studies demonstrate that pre-emergence herbicides even in combination with late sowing did not guarantee sufficient reduction of *A. myosuroides* in every year. In high density populations, average weed control efficacy of 95% is necessary to prevent the extension of the soil seed bank (Melander, 1995). Therefore, cinmethylin and late sowing can only be part of a weed control strategy with multiple weed management tactics. Under the pressure of resistance development *A. myosuroides* control programs were developed that combine diverse autumn and spring applications of herbicides with different modes of action. One option is to combine pre-emergence herbicides with post-emergence herbicides that could increase weed control efficacy in the current study. Further and additional options are wide crop rotation including autumn sown ~~crop~~ and spring crops, inversion tillage, false seed-bed preparation, stubble tillage, cover crops and competitive crop cultivars (Lutman *et al.*, 2013; Gerhards *et al.*, 2016; Travlos *et al.*, 2020; Schappert *et al.*, 2018).

In conclusion, the present study could demonstrate the benefit of cinmethylin as a new component of integrated weed control with a new mode of action. It significantly reduced *A. myosuroides* densities and saved grain yields, which is an indicator for high efficacy and selectivity of the new herbicides under variable soil conditions. Particular value of cinmethylin may lay in the fact that the molecule offers access to an additional mode of action that has not yet been exposed to the selection for resistance. It adds to the desirable diversity of options available to compose effective management programs for the challenging task of *A. myosuroides* control in winter cereals.

2.3.6. REFERENCES

BAILLY GC, DALE RP, ARCHER SA, WRIGHT DJ & KAUNDUN SS (2012) Role of residual herbicides for the management of multiple herbicide resistance to ACCase and ALS inhibitors in a black-grass population. *Crop Protection* **34,** 96–103.

BLAIR AM, CUSSANS JW & LUTMAN PJ, eds (1999) A biological framework for developing a weed management support system for weed control in winter wheat: weed competition and time of weed control. Brighton Crop Protection Conference Weeds, Brighton.

CAMPE R, HOLLENBACH E, KÄMMERER L et al. (2018) A new herbicidal site of action: Cinmethylin binds to acyl-ACP thioesterase and inhibits plant fatty acid biosynthesis. *Pesticide Biochemistry and Physiology* **148,** 116–125.

DAYAN FE (2003) Current Status and Future Prospects in Herbicide Discovery. *Plants (Basel, Switzerland)* **8,** 65.

DÉLYE C, MENCHARI Y, GUILLEMIN J-P et al. (2007) Status of black grass (Alopecurus myosuroides) resistance to acetyl-coenzyme A carboxylase inhibitors in France. *Weed Research* **47,** 95–105.

DROBNY HG, SALAS M & CLAUDE J (2006) Management of metabolic resistant black-grass (Alopecurus myosuroides Huds.) populations in Germany-challenges and opportunities. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **20,** 65.

DÜCKER R, ZÖLLNER P, PARCHARIDOU E, RIES S, LORENTZ L & BEFFA R (2019) Enhanced metabolism causes reduced flufenacet sensitivity in black-grass (Alopecurus myosuroides Huds.) field populations. *Pest management science* **75,** 2996–3004.

GERHARDS R, DENTLER J, GUTJAHR C, AUBURGER S & BAHRS E (2016) An approach to investigate the costs of herbicide-resistant Alopecurus myosuroides. *Weed Research* **56,** 407–414.

HAMMERTON JL (1967) Environmental Factors and Susceptibility to Herbicides. *Weeds* **15,** 330.

HARKER KN (2013) Slowing weed evolution with integrated weed management. *Canadian Journal of Plant Science* **93,** 759–764.

HEAP I (2014) Global perspective of herbicide-resistant weeds. *Pest management science* **70,** 1306–1315.

KUDSK P & KRISTENSEN JL, eds (1992) Effect of environmental factors on herbicide performance. Weed Science Society of Victoria, Victoria, Australia.

LUTMAN PJW, MOSS SR, COOK S & WELHAM SJ (2013) A review of the effects of crop agronomy on the management of Alopecurus myosuroides. *Weed Research* **53,** 299–313.

MELANDER B (1995) Impact of drilling date on Apera spica-venti L. and Alopecurus myosuroides Huds, in winter cereals. *Weed Research* **35,** 157–166.

MENEGAT A & NILSSON ATS (2019) Interaction of Preventive, Cultural, and Direct Methods for Integrated Weed Management in Winter Wheat. *Agronomy* **9,** 564.

MENNE HJ & HOGREFE C (2012) Impact of multiple resistance mechanisms in black-grass (Alopecurus myosuroides Huds.) populations on the of cereal herbicides. Julius-Kühn-Archiv; 434 (1); 65-74 / Julius-Kühn-Archiv; 434 (1); 65-74. *Julius-Kühn-Archiv* **1,** 65–74.

MENNE HJ, LABER B, KERLEN D & BEFFA R (2012) Effectiveness of flufenacet in controlling resistant black-grass (Alopecurus myosuroides Huds.) – comparison of glasshouse and field trial results. Julius-Kühn-

Archiv; 434 (1); 401-408 / Julius-Kühn-Archiv; 434 (1); 401-408. *Julius-Kühn-Archiv* **1,** 401–408.

MOSS S (2017) Black-grass (Alopecurus myosuroides): Why has this Weed become such a Problem in Western Europe and what are the Solutions? *Outlooks on Pest Management* **28,** 207–212.

MOSS SR, ed (1990) The seed cycle of Alopecurus myosuroides in winter cereals: a quantitative analysis. European Weed Research Society, Helsinki, Finland.

MOSS SR & CLARKE JH (1994) Guidelines for the prevention and control of herbicide-resistant black-grass (Alopecurus myosuroides Huds.). *Crop Protection* **13,** 230–234.

NEVE P (2007) Challenges for herbicide resistance evolution and management: 50 years after Harper. *Weed Research* **47,** 365–369.

ROBINSON MA, LETARTE J, COWBROUGH MJ, SIKKEMA PH & TARDIF FJ (2015) Winter wheat (Triticum aestivum L.) response to herbicides as affected by application timing and temperature. *Canadian Journal of Plant Science* **95,** 325–333.

SCHAPPERT A, MESSELHÄUSER M, SAILE M, PETEINATOS G & GERHARDS R (2018) Weed Suppressive Ability of Cover Crop Mixtures Compared to Repeated Stubble Tillage and Glyphosate Treatments. *Agriculture* **8,** 144.

TRAVLOS I, GAZOULIS I, KANATAS P, TSEKOURA A, ZANNOPOULOS S & PAPASTYLIANOU P (2020) Key Factors Affecting Weed Seeds' Germination, Weed Emergence, and Their Possible Role for the Efficacy of False Seedbed Technique as Weed Management Practice. *Frontiers in Agronomy* **2,** 1061.

2.4. Exploring the effects of different stubble tillage practices and glyphosate application combined with the new soil residual herbicide cinmethylin against *Alopecurus myosuroides* Huds. in winter wheat

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2.4.1. ABSTRACT

An effective control of *Alopecurus myosuroides* Huds. solely by a chemical treatment is not guaranteed anymore because populations exhibit resistance to almost all herbicide modes of action. Integrated weed management against black-grass is necessary to maintain high weed control efficacies in winter cereals. Four field experiments were conducted in Southwest Germany from 2018 until 2020 to control *A. myosuroides* with a combination of cultural and chemical methods. Stubble treatments including flat-, deep-, inversion soil tillage, false seedbed preparation and glyphosate use were combined with the application of the new pre-emergence herbicide cinmethylin in two rates in winter wheat. Average densities of *A. myosuroides* in the untreated control plots were up to 505 plants m^{-2}. The combination out of different stubble management strategies and the pre-emergence herbicide cinmethylin controlled 86-97% at the low rate and 95-100% of *A. myosuroides* plants at the high rate until 120 days after sowing. The different stubble tillage practices varied in their efficacy between trials and years. Most effective and consistent were pre-sowing glyphosate application on the stubble and stale seedbed preparation with a disc harrow. Stubble treatments increased winter wheat density in the first year but had no effect on crop density in the second year. Pre-emergence applications of cinmethylin did not reduce winter wheat densities. Multiple tactics of weed control including stubble treatments and pre-emergence application of cinmethylin provided higher and more consistent control of *A. myosuroides.* Integration of cultural weed management could prevent the herbicide resistance development.

KEYWORDS

Black-grass, soil tillage, herbicide resistance, integrated weed management, glyphosate, mode of action

2.4.2. INTRODUCTION

Since the 1990s there have been no new modes of action of herbicides. Therefore, the number of available, effective herbicides to combat herbicide resistant weed populations is strongly limited (Lutman et al., 2013; Chauvel et al., 2009; Rüegg et al., 2007). *Alopecurus myosuroides* Huds (black-grass) is a very abundant grass-weed in Western European cropping systems (Moss et al., 2007). The increasing density of *A. myosuroides* can be attributed to higher proportions of autumn sown crops such as winter cereals in the crop rotations, reduced tillage practices and a selection for herbicide resistant populations against all common mode of actions Chauvel et al., 2001; Menegat & Nilsson, 2019; Lutman et al., 2013; Délye et al., 2013; Heap, 2014).

The seeds of *A. myosuroides* are viable for up to 5 years in soil, whereby each year approximately 74% of the seeds in the soil are degraded (Moss, 2017; Moss, 1985). The main germination period of *A. myosuroides* is in September and October when most winter annual cereals are sown in Western Europe (Moss, 2017). Densities of around 100 *A. myosuroides* plants per m-2 cause grain yield losses in winter wheat of 20% (Gerhards et al., 2016). If not sufficiently controlled, populations can rapidly increase to densities of more than 1.000 plants per m-2 (Zeller et al., 2018). To prevent population increase, a control efficacy of min. 95% is required (Moss, 2017).

Many *A. myosuroides* populations in Europe are resistant to post-emergence herbicides, in particular to acetyl CoA carboxylase (ACCase) inhibitors and acetolactate synthase (ALS) inhibitors (Heap, 2014; Menne & Hogrefe, 2012). Soil residual herbicides are less affected by resistance since these active ingredients have been used less frequently (Bailly et al., 2012). Among the most frequently used soil residual herbicides in European winter cereal production are prosulfocarb, flufenacet (HRAC K3/15), pendimethalin (HRAC K1/3) and diflufenican (HRAC F1/12) (Bailly et al., 2012).

Nevertheless, recent studies have confirmed resistance to flufenacet in several populations of *A. myosuroides* (Dücker et al., 2019; Klingenhagen, 2012).

Herbicides are the dominant and often the most economically effective tool to control weeds in modern agriculture systems. It is intended to introduce cinmethylin as a new soil residual herbicide to control *A. myosuroides* and other grass-weeds in European winter cereals. The mode of action of cinmethylin was identified in 2018 (Campe et al., 2018). It inhibits the fatty acid thioesterases (FAT) in the plastid, which so far has not been identified as herbicide target. Cinmethylin had been traded by the Shell chemical company until 1989 as a pre-emergence herbicide to control grass-weeds in rice (Dayan, 2003). Since cinmethylin with its specific new mode of action has not been applied so far in European cropping systems, it is assumed *that A. myosuroides* populations are still sensitive to cinmethylin (Messelhäuser et al., 2021b).

However, for a sustainable use of a new mode of action like cinmethylin integrated weed management (IWM) practices should be applied. To achieve weed control levels of 95%, IWM strategies that combine preventive, non-chemical, and chemical measures are needed. Preventive methods like stubble tillage optionally supplemented with non-selective herbicide treatments efficiently controlled weeds and volunteers in winter cereals (Schappert et al., 2018). The timing, intensity and frequency strongly influenced the efficacy of stubble treatments (Pekrun & Claupein, 2006; Lutman et al., 2013). Inversion tillage by plough displaces the seeds vertically into deeper soil layers of up to 30 cm (Chauvel et al., 2001; Moss, 1980). Weed control efficacies of up to 69% can be achieved by lethal germination (Lutman et al., 2013). Shallow tillage to a depth of maximum 5 cm was very effective against annual grass-weeds including *A. myosuroides.* Seeds with no or a short period of primary dormancy were induced to germinate shortly after tillage. Emerged seedlings could then be removed by seedbed preparation (Melander & Rasmussen, 2000; Bond & Grundy, 2001).

The objective of this study was to test the efficacy of the new soil residual herbicide cinmethylin against *A. myosuroides* and the crop response at different locations over two years in winter wheat combined with different stubble treatments. The main hypotheses were that (i) both application rates of cinmethylin provide control efficacies of more than 80% against *A. myosuroides* until BBCH 30 in winter wheat. (ii) the combination of 375 g a.i. cinmethylin with different stubble management strategies achieves similar control efficacies than the application of 495 g a.i. cinmethylin, (iii) pre-emergence application of cinmethylin in combination with a mechanical stubble treatment achieve similar control efficacy of *A. myosuroides* as a sequence with a pre-sowing glyphosate treatment in winter wheat. Further it was hypothesized that (iv) cinmethylin shows high selectivity and does not reduce winter wheat grain yield.

2.4.3. MATERIALS AND METHODS

2.4.3.1. Experimental sites

Four field experiments were conducted in winter wheat in the Southwestern Germany over two seasons from October 2018 until august 2020. The four field experiments were located on a conventional farm site in Hirrlingen. From september 2018 until august 2019 two field experiments were conducted at Binsen (48.2522 °N, 8.5315 °E) and Risp (48.2506 °N, 8.5348 °E) and from september 2019 until august 2020 at Sieben Jauchert (48.2525 °N, 8.5342 °E) and B. Kreuz (48.2526 °N, 8.5356 °E). Average monthly temperatures and precipitation in comparison to the long-term means from 2018 until 2020 are shown in Table 2.4.3-1. The average annual temperatures were approximately 2 °C higher than the long-term average in both years. The autumn in 2018 and 2019 was extremely dry, the annual temperature was exceeded by 3 °C. The soil properties of the experiments were similar with a 300 mm parabrown top soil layer (Table 2.4.3-2).

Table 2.4.3-1: Mean temperature and precipitation for the region of Hirrlingen, Baden-Württemberg from September 2018 until August 2020. Long-term means for temperature and precipitation were assessed over the period from 1911 – 2010.*

	Mean temperatures (°C)				Mean precipitation (mm)			
	2018	2019	2020	Long term average	2018	2019	2020	Long term average
Jan		0.3	3.6	0.1		46.6	13.2	45.0
Feb		5.3	6.1	0.9		18.0	89.5	42.0
March		7.5	6.5	4.5		40.6	40.6	56.0
April		10.3	13.0	8.1		31.4	6.1	64.0
May		11.5	13.7	12.7		116.4	45.2	104.0
June		20.1	17.0	15.8		75.9	96.1	96.0
July		20.4	20.1	17.9		85.3	35.3	101.0
Aug		19.6	20.5	17.4		79.7	106.6	80.0
Sep	16.7	15.4		13.4	20.6	36.5		66.0
Oct	11.8	12.1		9.3	24.5	62.1		68.0
Nov	5.8	5.2		4.0	15.4	36.3		56.0
Dec	3.6	4.0		1.2	55.4	37.4		61.0

**source (“Wetter-bw,” 2020)*

Table 2.4.3-2: Soil properties of the four experimental locations.

Location	C_{org} % in DM	P mg P_2O_5	Clay %	Sand %	Silt %
Risp	1.94	45.36	27	4.2	68.7
Binsen	1.58	13.93	23.8	3.7	72.5
B. Kreuz	1.95	13.10	31.7	3.3	64.9
Sieben Jauchert	1.67	10.42	23.4	3.7	72.9

2.4.3.2. Experimental design

The experiments were set up as a two-factorial split-plot design with three repetitions. The plot size of all experiments was 36 m², while each plot measured 3 m in width and 12 m in length. The first factor was the stubble treatment in the period between the harvest of the previous crop and the seeding of winter wheat, containing nine (Binsen, Risp) and ten (B. Kreuz, Sieben Jauchert) treatments (Table 2.4.3-3). The second factor was the weed control method, including a pre-emergence application of LUXINUM® (495 g a.i. l^{-1} cinmethylin, BASF SE, Germany) in winter wheat with the full recommended field rate of 0.66 l ha^{-1} (495 g a.i. ha^{-1}) and a reduced application rate of 0.5 l ha^{-1} (375 g a.i. ha^{-1}). In 2019 the rate of 0 g a.i. ha^{-1} cinmethylin was added as a third application rate. In 2018, one treatment remained unsprayed (CON). The application dates were adapted to the individual years. Pre-emergence herbicide cinmethylin was applied 5 days after sowing (DAS) in BBCH 10 – 11 of the crops, with a field sprayer (Rau OHG Machinenfabrik, D2, 12 m, Kirchheim, Germany). Seedbed preparation in all treatments was done by a rotary hoe. Seedbed quality differed between the years according to the weather conditions in autumn. In 2018, seedbed was extremely rough with usable field capacity < 30% at the time of herbicide application, afterwards soil moisture was increased by sufficient rain falls.

Because of dry soil conditions at time of winter wheat sowing in 2018, 5 mm water was added with a boom sprayer directly after pre-emergence herbicide application. In spring, broadleaved weed species were controlled in all plots with Biathlon 4D (714 g a.i. kg^{-1} tritosulfuron, 54 g a.i. kg^{-1} florasulam, BASF SE, Germany).

Table 2.4.3-3: Conducted stubble treatments and dose of herbicide application.

			2018/2019		2019/2020	
Stubble Treatment		Time of soil tillage (DAH = days after harvest)	Binsen	Risp	B. Kreuz	Sieben Jauchert
			375; 495 g a.i. ha^{-1} cinmethylin		0; 375; 495 g a.i. ha^{-1} cinmethylin	
CON	Weed fallow without weed management	-	x	x	-	-
HERB	Only cinmethylin application without soil tillage	-	x	x	-	-
No-till+HERB	No soil tillage with chemical weed management	-	-	-	x	x

FST	Flat soil tillage with rotary harrow (5 cm)	14, 35, 50	x	x	x	x
DST	Deep soil tillage with wing share cultivator (15-16 cm)	14, 35, 50	x	x	x	x
PL	Turning soil tillage with plough (25 cm)	14	x	x	x	x
1x Gly	Single glyphosate treatment (4 l ha^{-1})	35	x	x	x	x
2x Gly	Dual Glyphosate treatment (4 l ha^{-1})	14, 50	x	x	x	x
SH	Single time straw harrow (0.5 -1 cm)	14	x	x	x	x
DH	Disc harrow (7-8 cm)	14, 35, 50	x	x	x	x
FSB + m	False seedbed (flat soil tillage + rotary harrow	14, 70	-	-	x	x

FSB + Gly	False seedbed (flat soil tillage) + single glyphosate	14, 70	-	-	x	x

2.4.3.3. Data collection

To reduce possible border effects, all measurements were performed only in the 10 center rows. Density of *A. myosuroides* was determined 56 and 120 days after sowing (DAS). The first date represents the end of the growing season in the year of sowing and the second date corresponds to the end of tillering of winter wheat. Winter wheat density was measured 120 DAS. Within each plot, A. *myosuroides* and winter wheat plants were counted with in a frame of 1/10 m^2 at four randomly chosen spots. Grain yield (t ha^{-1}) was measured by harvesting the centre of each plot at a size of 1.5 m x 12 m to exclude margin effects. Harvest was done by a plot harvester (Wintersteiger, Elite 3, Ried im Innkreis, Austria). Because of a hail event in 2019, harvest of the grain yield could only be performed at the experimental sites of B. Kreuz and Sieben Jauchert. Grain weights were transformed to a homogenous water content of 14%.

2.4.3.4. Statistical analysis

The data were analyzed with the statistical software R Studio (Version 3.6.2, RStudio Team, Boston, MA, USA). A linear mixed model was used to evaluate the response of weed density (plants m^{-2}), winter wheat density (tillers m^{-2}) and winter wheat grain yield (t ha^{-1}) to the examined factors (stubble treatment, weed control method) and interactions in the field experiments. Prior to the analysis, the data were visually checked for variance homogeneity and normal distribution of residuals. Results were square root transformed to homogenize variances and to normalize the distribution. In the results section, back transformed means are shown. An analysis of variance (ANOVA) was performed at $p \leq 0.05$. Stubble treatment and weed control method and all interactions between these variables were considered fixed effects. Multiple mean comparison tests were performed using the Tukey-HSD test at a significance level of $\alpha \leq 0.05$.

2.4.4. RESULTS

2.4.4.1. *A. myosuroides* densities 56 and 120 DAS

At the experimental site of Binsen, no significant differences in *A. myosuroides* density 56 days after seeding (DAS) could be detected. In average 53 *A. myosuroides* plants m^{-2} were measured in the untreated control plots (CON). In spring, 120 DAS infestations rates of *A. myosuroides* showed significant interaction between the stubble management strategies and the two herbicides rates. Densities varied in the CON of up to 505 plants m^{-2} at Binsen (Table 2.4.4-1). At the experimental site of Risp, no significant differences in *A. myosuroides* density 56 DAS could be detected. Infestation rates with *A. myosuroides* of 50 plants m^{-2} were observed in the CON. In spring, 120 DAS, significant interaction between the stubble management strategies and the two herbicide rates were observed. In average 302 plants m^{-2} were detected in the CON (Table 2.4.4-2).

At the experimental site of B. Kreuz, 56 DAS significant interaction between the stubble management strategies and the herbicide rate could be detected. Infestation rates of 19 plants m^{-2} in the CON+No-till were measured. At the experimental site of Sieben Jauchert no significant differences between the three application rates could be detected. In average, infestations rates with *A. myosuroides* ranged on a lower level with a maximum of 7 plants m^{-2} in CON+No-till. In autumn, 120 DAS infestations rates of *A. myosuroides* varied between 1 and 40 plants m^{-2} in the CON+No-till at B. Kreuz and Sieben Jauchert (Table 2.4.4-3). Significant differences between the three herbicide rates were observed with increasing weed control efficacy at higher application rates.

Table 2.4.4-1: A. myosuroides density (plants m^{-2}) 56 DAS and 120 DAS at Binsen. Means with the same letter are not significantly different according to HSD-test at $p \leq 0.05$. Levels of significance are shown behind the result for each experimental site separately. CON = untreated control, HERB = only cinmethylin application, 1x Gly = 1 x glyphosate application, 2x Gly =2 x glyphosate application, FST = Flat soil tillage, DST = deep soil tillage, PL = ploughing, DH = disc harrow, SH = straw harrow.

Treatment	**56 DAS**	**120 DAS**
CON	53.3 (± 24.4)	505.0 (± 58.4) d
HERB	12.8 (± 13.2)	46.7 (± 39.9) c
1x Gly	13.9 (± 28.9)	16.2 (± 14.4) ab
2x Gly	25.6 (± 33.3)	20.0 (± 29.0) ab
FST	3.9 (± 6.1)	24.2 (± 24.7) bc
DST	19.4 (± 27.5)	11.2 (± 17.5) ab
PL	20.6 (± 33.2)	6.7 (± 9.6) a
DH	10.0 (± 11.9)	22.9 (± 23.5) bc
SH	9.4 (± 7.3)	15.4 (± 23.6) ab

Table 2.4.4-2: A. myosuroides density (plants m^{-2}) 56 DAS and 120 DAS at Risp. Means with the same letter are not significantly different according to HSD-test at $p \leq 0.05$. Levels of significance are shown behind the result for each experimental site separately. Means with different small letters show significant differences within the herbicide rate of 375 g a.i. ha^{-1} according to Tukey HSD test at $\alpha \leq 0.05$. Means with different capital letters show significant differences within the herbicide rate of 495 g a.i. ha^{-1} according to Tukey HSD test at $\alpha \leq 0.05$. CON = untreated control, HERB = only cinmethylin application, 1x Gly = 1 x glyphosate application, 2x Gly =2 x glyphosate application, FST = Flat soil tillage, DST = deep soil tillage, PL = ploughing, DH = disc harrow, SH = straw harrow.

Treatment	**Herbicide rate (g a.i. ha^{-1})**	**56 DAS**	**120 DAS**
CON	0	50 (± 22.9)	302 (± 45.3)
HERB	375	0 (± 0)	9.2 (± 9.9) b
1x Gly	375	1.0 (± 3.3)	4.2 (± 5.1) ab
2x Gly	375	1.0 (± 3.3)	4.2 (± 9.9) ab
FST	375	0 (± 0)	9.2 (± 13.8) ab
DST	375	4.0 (± 7.3)	1.0 (± 2.9) a
PL	375	7.8 (± 10.9)	1.0 (± 2.9) a
DH	375	2.2 (± 6.7)	2.5 (± 4.5) ab
SH	375	0 (± 0)	3.3 (± 4.9) ab
HERB	495	0 (± 0)	0 (± 0) A
1x Gly	495	0 (± 0)	0 (± 0) A
2x Gly	495	1.0 (± 3)	0 (± 0 9A
FST	495	1.0 (± 1)	0.8 (± 2.9) A
DST	495	0 (± 0)	0 (± 0) A
PL	495	2.2 (± 6.7)	0 (± 0) A
DH	495	1.0 (± 3.3)	0 (± 0) A
SH	495	0 (± 0)	0 (± 9 A

Table 2.4.4-3: A. myosuroides density (plants m^{-2}) 56 DAS and 120 DAS at B. Kreuz and Sieben Jauchert. Means with the same letter are not significantly different according to HSD-test at $p \leq 0.05$. Levels of significance are shown behind the result for each experimental site separately. Means with different small letters show significant differences within the herbicide rate of 0 g a.i. ha^{-1} according to Tukey HSD test at $\alpha \leq 0.05$. Means with different capital letters show significant differences within the herbicide rate of 375 g a.i. ha^{-1} according to Tukey HSD test at $\alpha \leq 0.05$. Means with different bold small letters show significant differences within the herbicide rate of 495 g a.i. ha^{-1} according to Tukey HSD test at $\alpha \leq 0.05$. No-till+HERB = No soil tillage with chemical weed management, 1x Gly = 1 x glyphosate application, 2x Gly =2 x glyphosate application, FST = Flat soil tillage, DST = deep soil tillage, PL = ploughing, DH = disc harrow, SH = straw harrow, FSB + Gly = false seedbed + glyphosate, FSB + m = false seedbed + rotary hoe.

Treatment	Herbicide rate (g a.i. ha^{-1})	56 DAS		120 DAS	
		B. Kreuz	Sieben Jauchert	B. Kreuz	Sieben Jauchert
No-till+HERB	0	18.8 (± 25.2) b	6.7 (± 8.6)	40 (± 37.7) bcd	0.6 (± 51.0) bc
1x Gly	0	0 (± 0) a	8.9 (± 20.3)	8.9 (± 11.3) a	58.9 (± 60.0) abc
2x Gly	0	2.2 (± 4.4) a	4.4 (± 5.3)	15.6 (± 20.1) a	92.2 (± 83.9) c
FST	0	2.2 (± 4.4) a	2.2 (± 4.4)	45.6 (± 43.3) cde	52.8 (± 44.2) abc
DST	0	1.4 (± 4.0) a	4.3 (± 8.9)	81.1 (± 70.5) e	85 (± 74.3) bc
PL	0	1.1 (± 3.3) a	0 (± 0)	71.7 (± 55.8) de	71.7 (± 79.9) bc
DH	0	2.2 (± 6.7) a	4.4 (± 8.8)	44.4 (± 41.9) cde	31.1 (± 32.3) a
SH	0	0 (± 0) a	11.1 (± 16.9)	69.4 (± 50.2) de	72.8 (± 94.4) abc
FSB + Gly	0	1.1 (± 3.3) a	2.2 (± 4.4)	26.7 (± 23.8) abc	66.1 (± 58.3) bc
FSB + m	0	0 (± 0) a	4.4 (± 8.8)	16.7 (± 21.4) ab	56.1 (± 62.3) ab
No-till+HERB	375	0 (± 0) A	0 (± 0)	1.1 (± 3.2) A	68.9 (± 2.4) A
1x Gly	375	0 (± 0) A	0 (± 0)	2.2 (± 4.2) A	2.2 (± 7.3) A
2x Gly	375	1.1 (± 3.3) A	0 (± 0)	1.7 (± 5.1) A	1.7 (± 5.1) A

FST	375	4.4 (± 7.3) A	0 (± 0)	2.8 (± 7.5) A	0.6 (± 2.4) A
DST	375	0 (± 0) A	0 (± 0)	13.3 (± 28.5) A	1.7 (± 74.3) A
PL	375	4.4 (± 8.8) A	0 (± 0)	8.3 (± 18.6) A	5.6 (± 14.6) A
DH	375	0 (± 0) A	0 (± 0)	3.9 (± 12.0) A	1.1 (± 3.2) A
SH	375	2.2 (± 6.7) A	0 (± 0)	2.8 (± 5.8) A	2.8 (± 5.7) A
FSB + Gly	375	0 (± 0) A	0 (± 0)	3.3 (± 8.4) A	3.9 (± 12.4) A
FSB + m	375	1.1 (± 3.3) A	0 (± 0)	13.9 (± 45.5) A	1.1 (± 3.2) A
No-till+HERB	495	0 (± 0) **a**	0 (± 0)	3.3 (± 9.7) **a**	3.5 (± 12.2) **a**
1x Gly	495	1.1 (± 3.3) **a**	0 (± 0)	3.9 (± 12.4) **a**	5 (± 16.5) **a**
2x Gly	495	0 (± 0) **a**	0 (± 0)	0 (± 0) **a**	1.7 (± 3.8) **a**
FST	495	0 (± 0) **a**	0 (± 0)	9.4 (± 17.3) **a**	0 (± 0) **a**
DST	495	1(± 4.0) **a**	0 (± 0)	3.9 (± 28.5) **a**	1.1 (± 3.23) **a**
PL	495	1.1 (± 3.3) **a**	0 (± 0)	8.9 (± 20.5) **a**	0 (± 0) **a**
DH	495	0 (± 0) **a**	0 (± 0)	1.7 (± 3.8) **a**	0.5 (± 2.4) **a**
SH	495	0 (± 0) **a**	0 (± 0)	1.1 (± 3.2) **a**	0 (± 0) **a**
FSB + Gly	495	0 (± 0) **a**	0 (± 0)	2.7 (± 7.5) **a**	0 (± 0) **a**
FSB + m	495	0 (± 0) **a**	0 (± 0)	0.5 (± 2.4) **a**	2.7 (± 6.7) **a**

2.4.4.2. *A. mysuroides* control efficacy

Within the experimental year 2019, at the experimental site of Binsen and Risp overall high control efficacies of 80% could be achieved. Different stubble treatments followed by a pre-emergence application of cinmethylin resulted in increased, but not always significant differences in the overall control. In autumn, 56 DAS 100% control efficacy was observed in the flat soil tillage treatment (FST). Within the plots of double glyphosate application (2x Gly), deep soil tillage (DST) and ploughing (PL) reduced control efficacies of 64%-72% were detected (Figure 2.4.4-1a). In spring, 120 DAS no differences between the stubble management strategies were visible. All stubble management strategies achieved 99% of control efficacy, regardless of herbicide rate (Figure 2.4.4-1b). At the experimental site of Risp, 56 DAS the FST treatment reduced *A. myosuroides* density by 100%. Reduced control efficacies of 93% and 87% were observed in the treatments of DST and PL, respectively (Figure 2.4.4-1c). Similarly, at this experimental site, all stubble management strategies were able to achieve a control efficacy of 99% at 120 DAS (Figure 2.4.4-1d).

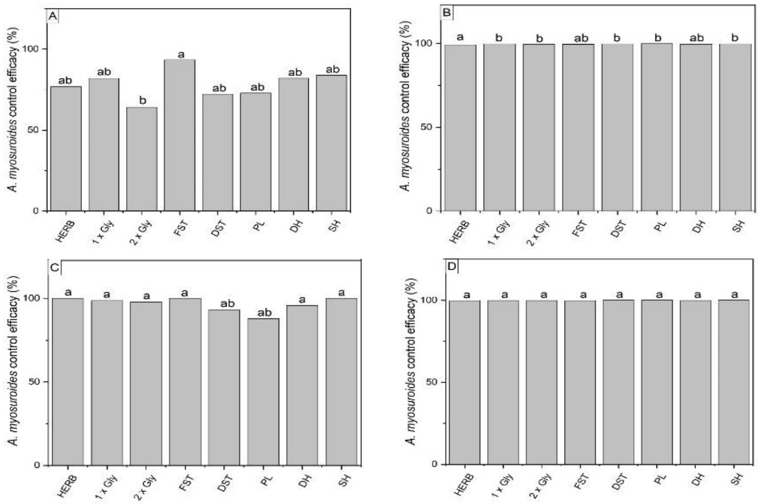

Figure 2.4.4-1: A. myosuroides control efficacy recorded at Binsen 56 DAS (A), Binsen 120 DAS (B), Risp 56 DAS (C), Risp 120 DAS (D) from 2019 - 2020. The application rate of cinmethylin is included in the upper right corner. Means with different letters show significant differences between the treatments according to Tukey HSD test at α ≤ 0.05. CON = untreated control, HERB = only cinmethylin application, 1x Gly = 1 x glyphosate application, 2x Gly =2 x glyphosate application, FST = Flat soil tillage, DST = deep soil tillage, PL = ploughing, DH = disc harrow, SH = straw harrow.

At the experimental site of B. Kreuz, 56 DAS significant interaction between herbicide rate and stubble management strategy were observed. Highest control efficacy of > 94% were achieved by all stubble management strategies in combination with a cinmethylin rate of 495 g a.i. The *A. myosuroides* control efficacy decreased at cinmethylin rate of 375 g a.i. ha^{-1} and 0 g a.i. ha^{-1}, respectively. All stubble management strategies within the application dose of 375 g a.i. achieved control efficacies of > 89% with the exception of FST (72%) and PL (79%). The stubble management strategies without herbicide application achieved control efficacies of > 89% with the exception of FST and 1x glyphosate (1x Gly) which showed a reduced efficacy of 83% (Figure 2.4.4-2a). In spring, 120 DAS no significant differences between the herbicide rates could be detected. Highest control efficacy of 87% was achieved by the treatment of false seedbed preparation followed by a rotary hoe (FSB + m). The poorest effect was observed for the stubble management strategy FST with 62% (Figure 2.4.4-2b). At the second experimental site in 2020, Sieben Jauchert significant differences between the herbicide rates were detected. On average across all stubble management strategies that had not been treated with cinmethylin, a 30% reduction in control efficacy was observed compared to the 375 g a.i. ha^{-1} and 495 g a.i. ha^{-1} rates 56 DAS (Figure 2.4.4-2c). In the subsequent year, 120 DAS a significant interaction between the herbicide rate and the stubble management strategy was detected. At this experimental site, big differences were visible between the cinmethylin-treated plots and the untreated plots. Within the cinmethylin rate of 495 g a.i. ha^{-1} control efficacies of > 87% were observed. Highest control efficacy of 100% was detected within the false seedbed preparation followed by a glyphosate application (FSB + Gly) and DST. A reduced control efficacy of 85% were observed by PL and FSB + m. Equally as at the experimental site of B. Kreuz, all stubble management strategies in combination with 375 g a.i. ha^{-1} achieved a control efficacy of minimum 89%. Whereby highest control efficacy of 99% was achieved by double glyphosate application (2x Gly) and FSB+m. Control efficacy of the stubble management strategies without cinmethylin treatment were less than 50%. The 2x Gly treatment with less than 10% performed worst while the DST treatment with almost 43% performed best (Figure 2.4.4-2d).

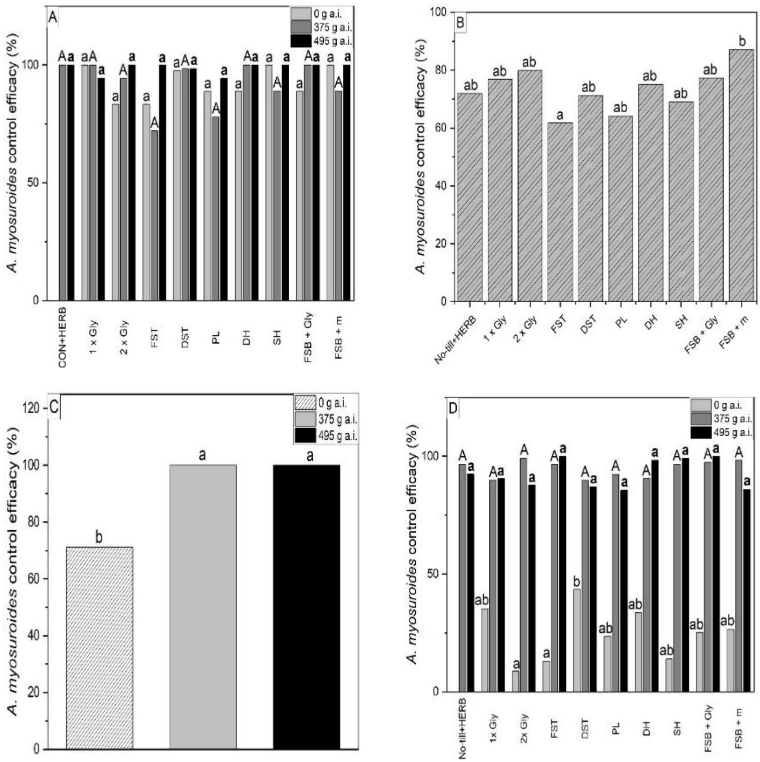

Figure 2.4.4-2: A. myosuroides control efficacy recorded at at B. Kreuz 56 DAS (A), B. Kreuz 120 DAS (B), Sieben Jauchert 56 DAS (C), Risp 120 DAS (D)from 2019 - 2020. The application rate of cinmethylin is included in the upper right corner. Means with different letters show significant differences between the treatments according to Tukey HSD test at α ≤ 0.05. No-till+HERB = No soil tillage with chemical weed management, 1x Gly = 1 x glyphosate application, 2x Gly =2 x glyphosate application, FST = Flat soil tillage, DST = deep soil tillage, PL = ploughing, DH = disc harrow, SH = straw harrow, FSB + Gly = false seedbed + glyphosate, FSB + m = false seedbed + rotary hoe.

2.4.4.3. Winter wheat densities in the field experiments

Pre-emergence herbicide application with cinmethylin had no phytotoxic effect on winter wheat density. Therefore, all dose rates of cinmethylin tested at the different location were pooled for the individual stubble treatments. The density of winter wheat was above 200 plants m^{-2} in all treatments except of the CON at the site Binsen und Risp (Table 2.4.4-4) were a significant lower density of 138-142 plants m^{-2} was counted. The highest densities of winter wheat plants were observed in PL plots with 288 and 281 plants m^{-2} for the sites at Binsen and Risp. For B. Kreuz and Sieben Jauchert, highest densities were observed with the 2x Gly application with 265 and 295 plants m^{-2}.

Table 2.4.4-4: Winter wheat density (plants m^{-2}) 120 DAS (Binsen, Risp, B.Kreuz and Sieben Jauchert). Means with the same letter are not significantly different according to HSD-test at $p \leq 0.05$. Levels of significance are shown behind the result for each experiment site separately. CON = untreated control, HERB = only cinmethylin application, No-till+HERB = No soil tillage with chemical weed management, 1 x Gly = 1 x glyphosate application, 2 x Gly =2 x glyphosate application, FST = Flat soil tillage, DST = deep soil tillage, PL = ploughing, DH = disc harrow, SH = straw harrow, FSB + Gly = false seedbed + glyphosate, FSB + m = false seedbed + rotary hoe.

	Winter wheat density (plants m^{-2})			
Treatments	**Binsen**	**Risp**	**B. Kreuz**	**Sieben Jauchert**
CON	138 a	142 a	-	-
HERB	209 b	220 b	-	-
No-till+HERB	-	-	258 a	286 a
1x Gly	254 cd	258 bc	244 a	290 a
2x Gly	276 cd	286 c	265 a	295 a
FST	270 cd	248 bc	256 a	282 a
DST	281 cd	269 bc	255 a	280 a
PL	288 d	276 bc	249 a	289 a
DH	236 bc	281 c	249 a	292 a
SH	242 bcd	220 b	249 a	286 a
FSB + Gly	-	-	258 a	298 a
FSB + m	-	-	264 a	280 a

2.4.4.4. Winter wheat grain yield in field experiments

According to the statistical analysis no significant interaction between the application rates of cinmethylin and the stubble treatments could be detected in regard to the grain yield. At the site of B. Kreuz, grain yield could not significantly increase due to stubble treatments compared to the untreated control. Between the stubble treatments at the site of Sieben Jauchert, no significant differences either between the application rates of cinmethylin neither of the stubble treatments could be observed (Table 2.4.4-5).

Table 2.4.4-5: Winter wheat grain yield (t ha^{-1}) in 2020 at B. Kreuz and Sieben Jauchert. Means with the same letter are not significantly different according to HSD-test at $p \leq 0.05$. Levels of significance are shown behind the result for each experiment site separately. No-till+HERB = No soil tillage with chemical weed management, 1 x Gly = 1 x glyphosate application, 2 x Gly =2 x glyphosate application, FST = Flat soil tillage, DST = deep soil tillage, PL = ploughing, DH = disc harrow, SH = straw harrow, FSB + Gly = false seedbed + glyphosate, FSB + m = false seedbed + rotary hoe.

Winter wheat grain yield (t ha^{-1})		
Treatments	**B. Kreuz**	**Sieben Jauchert**
No-till+HERB	7.7 c	8.4 a
1x Gly	7.5 bc	8.5 a
2x Gly	7.5 bc	8.8 a
FST	7.5 bc	8.6 a
DST	7.1 abc	8.4 a
PL	6.3 a	8.6 a
DH	6.8 abc	8.5 a
SH	6.5 ab	8.8 a
FSB + Gly	7.1 abc	8.8 a
FSB + m	7.0 abc	8.7 a

2.4.5. DISCUSSION

Both application rates of cinmethylin, 375 and 495 g ai ha^{-1}, could reduce *Alopecurus myosuroides* Huds. (black-grass) density by 86-97% and 95-

100%, respectively. Therefore, hypothesis (i) was proved in this study. High efficacy (98%) of 495 g ai ha^{-1} cinmethylin against *A. myosuroides* was also found in previous field studies (Messelhäuser et al., 2021a). A lower rate of 375 g ai ha^{-1} cinmethylin still provided 80% control efficacy against *A. myosuroides,* which corresponded well to the 85% weed control efficacy against *Lolium rigidum* L. (ryegrass) in Australian field studies (Busi et al., 2020). Those ryegrass populations in Australia showed already high level of resistance against trifluralin, indicating that cinmethylin is not affected by resistance to other herbicide modes of action.

Control efficacy of cinmethylin against *A. myosuroides* was similar to other pre-emergence herbicides commonly used in Western European winter wheat fields. In studies of (Bailly et al., 2012), pre-emergence application of flufenacet controlled 98% and 95–97% for the combination of flufenacet and pendimethalin. Flufenacet efficacy against *A. myosuroides* was slightly reduced to 75% in a previous study (Messelhäuser et al., 2021a). However, weed control efficacies of pre-emergence herbicides may differ from year to year under Variations in soil water content, limited soil persistence and a long period of the emergence of *A. myosuroides* from autumn until spring can explain the difference in weed control efficacy of pre-emergence herbicides (Kudsk & Kristensen, 1992). Furthermore, pre-emergence herbicides can be lost due to surface evaporation or leaching in wet soils (Kudsk & Kristensen, 1992; Hammerton, 1967). Sufficient soil moisture, temperatures and the very early developmental stages of the weeds at the time of herbicide application are necessary for high efficacies of pre-emergence herbicide use (Hammerton, 1967; Kudsk & Kristensen, 1992). In addition, factors such as herbicide dose, persistence, spraying accuracy, seedbed conditions and weed emergence patterns also influence the efficacy of a pre-emergence herbicide (Menne et al., 2012). Reduced control efficacies of pre-emergence herbicides were often observed under dry conditions and at high clay and organic matter contents (Akesson & Yates, 1987; Medd et al., 2001; Kudsk & Kristensen, 1992). Dry conditions, such as those experienced in the autumn of 2018, showed greater variation in the potential to create a fine and firm seedbed following the different stubble treatments. The persistence of pre-emergence herbicides in the soil is often insufficient to provide effective weed control until BBCH 30 of winter cereals (Kudsk & Kristensen, 1992).

Nevertheless, in the present study the reduced rate of 375 g a.i. ha^{-1} cinmethylin achieved similar control efficacies than the full rate of 495 g a.i. ha^{-1} cinmethylin. Thus, hypothesis (ii) can was also proved. Within both experimental years, *A. myosuroides* control efficacies of minimum 89% were achieved by the reduced cinmethylin rate in winter wheat without any stubble treatment. Also, other studies reported that a 50% dose of tralkoxydim consistently gave > 85% control of *Avena fatua* L. (wild oat) in barley (Hordeum vulgare L.) (Belles et al., 2000). An Australian study by Walker et al. (2002) found that the efficacy of clodinafop and tralkoxydim on wild oat (Avena ludoviciana Durieu.) and paradoxa grass (Phalaris paradoxa L.) was still adequate at 50-75% of the recommended doses. Nevertheless, reduced herbicide rates carry a high risk of inadequate weed control. Reduced herbicide rates might fasten the process of resistance development. During the last years, the use of reduced rates of herbicides has been associated with the increasing number of cases of non-target site resistance in grass species such as *A. myosuroides* and *Lolium ssp* (Kudsk, 2014). In cases where the least susceptible individuals in the population survive the use of reduced rates, this selection leads to a stepwise increase of the resistance level in the weed population. This is only valid if the use of reduced herbicide rates is due to lower efficacy, but not if high susceptibility of weed species present in the field or optimal conditions are the reasons for reducing herbicide rates (Kudsk, 2014). A high abundance in weed population increases the risk of selecting resistant weed biotypes because the probability of having resistant plants in the population increases with population size. One of the main purposes of integrated weed management (IWM) is to suppress problematic weed species using multiple tactics of weed control (Harker & O'Donovan, 2013; Kudsk, 2014). IWM is also implemented in the Green Deal targets and Farm-to-Fork (F2F) strategy, which was published in May 2020. The F2F aims to make the EU food system fair, healthy and environmentally friendly and was established as a cornerstone of the European Green Deal under the European Commission's program for the period 2019-2024. The Commission calls to reduce the overall use and risk of chemical pesticides by 50%. Combining the use of herbicides with other weed control methods (e.g., tillage, cover crops, crop rotation, competitive crops, high crop seed rates, reduced row spacing, specific fertilizer placement) reduce the risk of resistance development (Lemerle et al., 2001; Sarrantonio & Gallandt, 2003; Blackshaw et al., 2004; Zhang et al., 2000).

Preventive and cultural control measures before sowing of the main crop are elements of IWM, which shall reduce the plant density of *A. myosuroides* in the following crop, thereby supporting the control level of the subsequent herbicide application within the crop (Harker & O'Donovan, 2013). Stubble soil cultivation and/or glyphosate application, as used in this study, varied in their effect and supporting contribution to the control efficacy of cinmethylin. Most effective and consistent was the use of glyphosate (> 77%) and disc harrow (DH) with control efficiencies > 76% by BBCH 30 of the winter wheat. In the first experimental year, the FST treatment showed control efficacies of > 93% in spring. In the second experimental year both FSB treatments reached control efficacies of 77 – 100%. Therefore, the (iii) hypothesis that pre-emergence application of cinmethylin in combination with a mechanical stubble treatment achieve similar control efficacy of *A. myosuroides* as a sequence with a pre-sowing glyphosate treatment in winter wheat was also provedin this study. However, shallow tillage can be very effective against annual weeds because even *A. myosuroides* seeds with no or short dormancy are stimulated to germinate shortly after tillage. Seedlings that have already germinated could then be removed before sowing winter cereals (Moss & Clarke, 1994). The efficiency of the different strategies could be increased by applying the pre-emergence herbicide. The high control efficacy of the FSB treatments agrees with a study of Menegat & Nilsson (2019), whereby the combination of false seedbed preparation with an herbicide treatment in autumn achieved control efficacies of up to 85%. Nevertheless, the control efficacy of a pre-sowing glyphosate treatment is high, as expected. In contrast to finding of Schappert et al. (2018) also the 1x Gly achieved high control efficacies of up to 100%. However, the use of glyphosate has recently been under strong criticism and the future use in the EU after expiration of approval in December 2022 is open. Therefore, it is becoming increasingly important to investigate how cultural weed control measures are able to at least partially replace fall-to-spring glyphosate applications. Tendencies that were already visible in autumn became evident in spring. Further, this indicates a long-lasting persistence of the new pre-emergence herbicide.

In this study, cinmethylin did not damage winter wheat crops. Winter wheat densities were higher in the cinmethylin treatments compared to the untreated controls. Thus, hypothesis four was also confirmed. Within all four experiments crop seeds were placed at a depth of 3 cm. In an Australian study, a high degree of selectivity of cinmethylin was also observed, whereby wheat seeds were placed at a depth of 1 cm (Busi et al., 2020). As a result, no differences in seedling emergence were observed between the cinmethylin-treated and untreated control plots (Busi et al., 2020). According to this study, it has to be mentioned that crop damage due to the application of cinmethylin could happen if crop seeds are in direct contact with the herbicide. Conversely, this also increases the weed control effect. Selectivity and efficacy of cinmethylin will depend on the relative position of the crop and weed seeds, seedbed quality and absorption potential on clay minerals and soil organic matter.

2.4.6. CONCLUSIONS

In a two-year study on four experimental sites in Southwest Germany, it was demonstrated that cinmethylin provided high weed control efficacy and good selectivity in winter wheat. Stubble tillage practices and stubble applications of glyphosate resulted in additional weed control efficacy to the cinmethylin treatments. However, further investigations are required to test other non-chemical weed control treatments in combination with pre-emergence cinmethylin application. In order to maintain a sustainable use of cinmethylin for an effective in-crop *A. myosuroides* control, preventive and cultural weed control methods such as stubble tillage are important components of integrated weed management and resistance management.

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2.4.7. REFERENCES

AKESSON NB & YATES WE, eds (1987) Effect of weather factors on the application of herbicides, Champaign, IL, USA.

BAILLY GC, DALE RP, ARCHER SA, WRIGHT DJ & KAUNDUN SS (2012) Role of residual herbicides for the management of multiple herbicide resistance to ACCase and ALS inhibitors in a black-grass population. *Crop Protection* **34,** 96–103.

BELLES DS, THILL DC & SHAFII B (2000) PP-604 rate and Avena fatua density effects on seed production and viability in Hordeum vulgare. *Weed Science* **48,** 378–384.

BLACKSHAW RE, MOLNAR LJ & JANZEN HH (2004) Nitrogen fertilizer timing and application method affect weed growth and competition with spring wheat. *Weed Science* **52,** 614–622.

BOND W & GRUNDY AC (2001) Non-chemical weed management in organic farming systems. *Weed Research* **41,** 383–405.

BUSI R, DAYAN FE, FRANCIS I et al. (2020) Cinmethylin controls multiple herbicide-resistant Lolium rigidum and its wheat selectivity is P450-based. *Pest management science* **76,** 2601–2608.

CAMPE R, HOLLENBACH E, KÄMMERER L et al. (2018) A new herbicidal site of action: Cinmethylin binds to acyl-ACP thioesterase and inhibits plant fatty acid biosynthesis. *Pesticide Biochemistry and Physiology* **148,** 116–125.

CHAUVEL B, GUILLEMIN J, COLBACH N & GASQUEZ J (2001) Evaluation of cropping systems for management of herbicide-resistant populations of blackgrass (Alopecurus myosuroides Huds.). *Crop Protection* **20,** 127–137.

CHAUVEL B, GUILLEMIN J-P & COLBACH N (2009) Evolution of a herbicide-resistant population of Alopecurus myosuroides Huds. in a long-term cropping system experiment. *Crop Protection* **28.,** 343–349.

DAYAN FE (2003) Current Status and Future Prospects in Herbicide Discovery. *Plants (Basel, Switzerland)* **8**.

DÉLYE C, JASIENIUK M & LE CORRE V (2013) Deciphering the evolution of herbicide resistance in weeds. *Trends in genetics : TIG* **29,** 649–658.

DÜCKER R, ZÖLLNER P, PARCHARIDOU E, RIES S, LORENTZ L & BEFFA R (2019) Enhanced metabolism causes reduced flufenacet sensitivity in black-grass (Alopecurus myosuroides Huds.) field populations. *Pest management science* **75,** 2996–3004.

GERHARDS R, DENTLER J, GUTJAHR C, AUBURGER S & BAHRS E (2016) An approach to investigate the costs of herbicide-resistant Alopecurus myosuroides. *Weed Research* **56,** 407–414.

HAMMERTON JL (1967) Environmental Factors and Susceptibility to Herbicides. *Weeds* **15,** 330.

HARKER KN & O'DONOVAN JT (2013) Recent Weed Control, Weed Management, and Integrated Weed Management. *Weed Technology* **27,** 1–11.

HEAP I (2014) Global perspective of herbicide-resistant weeds. *Pest management science* **70,** 1306–1315.

KLINGENHAGEN G (2012) Comparison of different black-grass populations (Alopecurus myosuroides Huds.) in their susceptibility to herbicides under field conditions. Julius-Kühn-Archiv; 434 (1); 81-87 / Julius-Kühn-Archiv; 434 (1); 81-87.

KUDSK P (2014) Reduced herbicide rates: present and future. *Julius-Kühn-Archiv*, 37–44.

KUDSK P & KRISTENSEN JL, eds (1992) Effect of environmental factors on herbicide performance. Weed Science Society of Victoria, Victoria, Australia.

LEMERLE D, GILL GS, MURPHY CE et al. (2001) Genetic improvement and agronomy for enhanced wheat competitiveness with weeds. *Australian Journal of Agricultural Research* **52,** 527.

LUTMAN PJW, MOSS SR, COOK S & WELHAM SJ (2013) A review of the effects of crop agronomy on the management of Alopecurus myosuroides. *Weed Research* **53,** 299–313.

MEDD RW, VAN DE VEN RJ, DI PICKERING & NORDBLOM T (2001) Determination of environment-specific dose-response relationships for clodinafop-propargyl on Avena spp. *Weed Research* **41,** 351–368.

MELANDER & RASMUSSEN (2000) Reducing intrarow weed numbers in row crops by means of a biennial cultivation system. *Weed Research* **40,** 205–218.

MENEGAT A & NILSSON ATS (2019) Interaction of Preventive, Cultural, and Direct Methods for Integrated Weed Management in Winter Wheat. *Agronomy* **9,** 564.

MENNE HJ & HOGREFE C (2012) Impact of multiple resistance mechanisms in black-grass (Alopecurus myosuroides Huds.) populations on the of cereal herbicides. Julius-Kühn-Archiv; 434 (1); 65-74 / Julius-Kühn-Archiv; 434 (1); 65-74.

MENNE HJ, LABER B, KERLEN D & BEFFA R (2012) Effectiveness of flufenacet in controlling resistant black-grass (Alopecurus myosuroides Huds.) – comparison of glasshouse and field trial results. Julius-Kühn-Archiv; 434 (1); 401-408 / Julius-Kühn-Archiv; 434 (1); 401-408.

MESSELHÄUSER M, SAILE M, SIEVERNICH B & GERHARDS R (2021a) Effect of cinmethylin against Alopecurus myosuroides Huds. in winter cereals. *Plant, Soil and Environment* **67,** 46–54.

MESSELHÄUSER MH, LINN AI, MATHES A, SIEVERNICH B & GERHARDS R (2021b) Development of an Agar Bioassay Sensitivity Test in Alopecurus myosuroides for the Pre-Emergence Herbicides Cinmethylin and Flufenacet. *Agronomy* **11,** 1408.

MOSS S (2017) Black-grass (Alopecurus myosuroides): Why has this Weed become such a Problem in Western Europe and what are the Solutions? *Outlooks on Pest Management* **28,** 207–212.

MOSS SR (1980) A study of populations of black-grass (Alopecurus myosuroides) in winter wheat, as influenced by seed shed in the previous crop, cultivation system and straw disposal method. *Annals of Applied Biology* **94,** 121–126.

MOSS SR (1985) The survival of Alopecurus myosuroides Huds. seeds in soil. *Weed Research* **25,** 201–211.

MOSS SR & CLARKE JH (1994) Guidelines for the prevention and control of herbicide-resistant black-grass (Alopecurus myosuroides Huds.). *Crop Protection* **13,** 230–234.

MOSS SR, PERRYMAN SA & TATNELL LV (2007) Managing Herbicide-resistant Blackgrass (Alopecurus Myosuroides): Theory and Practice. *Weed Technology* **21,** 300–309.

PEKRUN C & CLAUPEIN W (2006) The implication of stubble tillage for weed population dynamics in organic farming. *Weed Research* **46,** 414–423.

RÜEGG WT, QUADRANTI M & ZOSCHKE A (2007) Herbicide research and development. Challenges and opportunities. *Weed Research* **47,** 271–275.

SARRANTONIO M & GALLANDT E (2003) The Role of Cover Crops in North American Cropping Systems. *Journal of Crop Production* **8,** 53–74.

SCHAPPERT A, MESSELHÄUSER M, SAILE M, PETEINATOS G & GERHARDS R (2018) Weed Suppressive Ability of Cover Crop Mixtures Compared to Repeated Stubble Tillage and Glyphosate Treatments. *Agriculture* **8,** 144.

WALKER SR, MEDD RW, ROBINSON GR & CULLIS BR (2002) Improved management of Avena ludoviciana and Phalaris paradoxa with more densely sown wheat and less herbicide. *Weed Research* **42,** 257–270.

ZELLER A, KAISER Y & GERHARDS R (2018) Suppressing Alopecurus myosuroides Huds. in Rotations of Winter-Annual and Spring Crops. *Agriculture* **8,** 91.

ZHANG J, WEAVER SE & HAMILL AS (2000) Risks and reliability of using herbicides at below-labeled rates. *Weed Technology* **14,** 106–115.

2.5. Effects of stubble treatments, delayed sowing and pre-emergence cinmethylin application on *Alopecurus myosuroides* Huds. density and cereal grain yield

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2.5.1. ABSTRACT

The use of pre-emergence herbicides in winter annual cereals often needs to be combined with other weed control tactics to provide sufficient weed control efficacy for the entire growing season. In the present study, the new pre-emergence herbicide cinmethylin for the control of *Alopecurus myosuroides* Huds. was tested in four field experiments in winter wheat and winter triticale in Southwestern Germany over a period of three years. It inhibits the fatty acid thioesterases (FAT) in the plastid and represent a new mode-of-action for chemical weed control. Treatments with cinmethylin were combined with stubble treatments and delayed drilling of winter annual cereals in a three-factorial block design. Average densities of *A. myosuroides* in the untreated control plots ranged from 38-1,233 plants m^{-2}. The pre-emergence herbicide cinmethylin controlled 58-99% of *A. myosuroides* plants until 120 days after sowing. Additive and synergistic effects of cinmethylin and delayed drilling were found for all studies reducing *A. myosuroides* density by more than 90%. Stubble treatments including one and two passes of chisel ploughing, chisel ploughing followed by glyphosate application and conservation stubble tillage with a straw harrow did not result in significant different *A. myosuroides* densities in the following winter cereal crops. Winter wheat and winter triticale grain yields were significantly increased by the use cinmethylin combined with delayed drilling. These data underline the benefits of integrated weed management using different weed control tactics.

KEYWORDS

Pre-emergence herbicide, Integrated weed management, preventive weed control

2.5.2. INTRODUCTION

Cinmethylin is a potential new pre-emergence herbicide to control *Alopecurus myosuroides* Huds. and other grass weeds in cereals and oil-seed rape. Cinmethylin had been developed by Shell chemical company in the 1980ies. It was used in Asia to control grass-weeds in rice (Dayan, 2003) but so far, it has not been applied in Europe. The mode of action of cinmethylin was recently identified by Campe et al. (2018). It inhibits the fatty acid thioesterases (FAT) in the plastid and represent a new mode of action for chemical weed control (HRAC-group: 30 (Q)). Already before cinmethylin had been registered for weed control in European winter wheat production, two out of 17 *A. myosuroides* populations with reduced sensitivity to pre-emergence herbicide flufenacet (Messelhäuser et al., 2021b) were also tolerant to recommended field rates of cinmethylin, even though flufenacet inhibits a different pathway in the fatty acid synthesis than cinmethylin. Repeated application of herbicides has rapidly selected for many resistant populations of *A. myosuroides* in Western Europe against almost all herbicide modes of action registered for *A. myosuroides* control. More populations were resistant to post-emergence herbicides inhibiting the photosystem II-(PS2), acetyl CoA carboxylase (ACCase)- and acetolactate synthase (ALS) than to pre-emergence herbicides (Drobny et al., 2006; Délye et al., 2007; Neve, 2007; Menne & Hogrefe, 2012; Menne et al., 2012; Bailly et al., 2012; Heap, 2014). However, it appears possible that *A. myosuroides* plants resistant to cinmethylin may also be selected during several seasons of cinmethylin use in winter annual crops and could then dominate the respective population. Integrated weed control measures shall be used to reduce that risk and to exert its efficacy potential over a longer period of time. *A. myosuroides* is prone for herbicide resistance because of several reasons. Population densities rapidly increased over the past decades due to a shift of cropping systems to higher proportions of winter annual crops and reduced tillage practices (Lutman et al., 2013; Gerhards et al., 2016). Selection pressure remains high because the same herbicides are often sprayed several times within a single rotation cycle (Zeller et al., 2018). Herbicide resistance can spread within agricultural fields and from field to field due to pollen movement by cross-pollination and seed transport with unclean equipment like combine harvesters, particularly if harvest is contracted to specialized service providers. Finally, seeds persist in the soil seed bank over a period of up to 8 years (Moss, 1990; Gerhards et al., 2016; Moss, 2017). For those reasons, *A. myosuroides* became a very problematic weed in Western European winter cereal production causing approximately 20% grain yield losses at densities of 100 plants per m^{-2} (Blair et al., 1999; Zeller et al., 2018; Zeller et al., 2021).

It is mostly agreed that only integrated weed control strategies including preventive and curative methods of weed control can successfully suppress *A. myosuroides* (Lutman et al., 2013; Moss, 2017). Pre-emergence herbicides are usually not sufficient to consistently reach critical level of minimum 95% weed control efficacy against *A. myosuroides*, which is required to prevent an increase of population densities (Melander, 1995; Menegat & Nilsson, 2019; Messelhäuser et al., 2021a). Among preventive methods, integration of spring crops in winter cereal rotations reduced *A. myosuroides* densities by up to 88%, inversion tillage by 69%, delayed autumn drilling by 50%, selection of competitive crop cultivars and increasing crop density by up to 40% (Moss & Clarke, 1994; Lutman et al., 2013; Zeller et al., 2018; Zeller et al., 2021). Cover cropping, non-selective herbicide application on the stubble and stubble tillage suppressed *A. myosuroides* densities in the fall-to-spring season by more than 90% (Schappert et al., 2018). Chisel ploughing is a common stubble tillage practice to incorporate residues of the previous crop. Although chisel ploughing results in better decomposition of crop residues and volunteer seeds than shallow stubble tillage operations, it can induce secondary dormancy in fresh *A. myosuroides* seeds (Moss, 2017). Inversion tillage with a moldboard plough and repeated shallow tillage operations (up to 5 cm depth) after harvesting the previous crop resulted in better *A. myosuroides* suppression than chisel ploughing. Inversion tillage induced many seeds to germinate in deep soil layers of approximately 20 cm but were not capable to emerge (lethal germination) (Gerhards et al., 2016). Shallow stubble tillage operations with a straw harrow prevented secondary dormancy and induced many seeds to germinate (Moss, 2017; Zeller et al., 2021). A second pass of shallow tillage controlled the emerged *A. myosuroides* seedlings (Moss, 2017; Menegat & Nilsson, 2019). All preventive weed control methods listed above were mostly investigated separately in the previous studies. Few studies have combined preventive and curative methods in multi-factorial experiments (Zeller et al., 2021). The objectives of this study were to determine the combined effects of stubble treatments, late drilling and the pre-emergence application of cinmethylin on *A. myosuroides* density and winter cereal grain yield. The hypotheses were that i) cinmethylin provided more than 80% weed control efficacy against *A. myosuroides* until the end of the vegetative growth stage (tillering) of winter cereals. The second hypothesis was that ii) delayed autumn drilling had an additive or synergistic effect on the efficacy of the pre-emergence cinmethylin application. It was further hypothesized that iii) repeated stubble tillage and a combination of chisel ploughing and non-selective herbicide application on the stubble reduced *A. myosuroides* in the following winter cereal compared to reduced stubble tillage.

2.5.3. MATERIALS AND METHODS

2.5.3.1. Experimental sites

Four fields experiments were conducted in winter wheat (2) and winter triticale (2) in Southwestern Germany from autumn 2017 until summer 2020. Three experiments were located at the research station Ihinger Hof (48°44'32.5"N 8°55'31.1"E) of the University of Hohenheim and one on a commercial farm site in Entringen 48°33'31.1"N 8°57'23.6"E). Climatical conditions were similar at both locations. Average monthly temperatures and precipitation during the experimental period and the long-term means are shown in Table 2.5.3-1. Temperatures were 1-2 °C above the long-term average during all three years. All years of the study were characterized by longer periods of drought in early spring, summer and autumn. In 2017 and 2019, soil was very dry before and shortly after sowing of winter cereals. The soil type at both locations was a parabrown soil containing 41% clay in Entringen and 32% clay at Ihinger Hof. Organic carbon contents ranged from 1.5% at Ihinger Hof to 2.1% in Entringen.

Table 2.5.3-1: Average monthly temperatures and precipitation at Ihinger Hof research station from October 2017 until August 2020 and long-term means from 1961 until 1990.*

	Mean temperatures (°C)					Mean precipitation (mm)				
	2017	2018	2019	2020	Long term average	2017	2018	2019	2020	Long term average
Jan		3.9	-0.9	2.3	-0.4		89.0	45.6	11.2	50.0
Feb		-2.4	3.5	4.8	0.7		19.4	13.1	88.2	45.0
March		2.9	6.1	4.7	4.0		21.2	47.1	49.7	51.3
April		12.4	8.6	10.9	7.9		17.4	26.7	4.8	60.1
May		14.9	10.1	11.9	12.2		75.1	107.2	45.6	80.1
June		17.4	18.5	15.5	15.5		32.5	52.2	85.4	92.6
July		19.9	18.7	18.3	17.5		32.0	53.9	15.3	67.5
Aug		19.6	18.2	19.3	16.8		28.8	82.4	11.2	73.6
Sep		14.8	13.7		13.6	5.8	78.0	28.4		57.2
Oct	10.3	10.1	10.8		9.0	51.1	26.4	53.6		45.2
Nov	4.0	4.5	3.9		3.7	63.0	19.5	43.4		62.0
Dec	1.1	2.6	2.7		0.7	32.5	83.9	37.4		53.3

**source (“Wetter-bw”, 2020)*

2.5.3.2. Experimental design

A three factorial randomized complete block design with three repetitions was realized in all four experiments. Each plot had a length of 12 m and a width of 3 m. The first factor was the **stubble treatment** between the harvest of the previous crop and the sowing of the winter cereal. Four treatments were tested (Table 2). Chisel ploughing was applied once shortly after harvest of the previous crop in the “reduced” treatment. In “repeated” treatment, chisel ploughing was done twice, shortly after harvest of the previous crop and four weeks later to provide better control of emerging weeds and crop volunteers than in the "reduced" treatment. "Conservation" tillage is often applied when problems with soil erosion occurs. It was conducted twice with a straw harrow. In one treatment, chisel ploughing was followed by one application of non-selective herbicides (Table 2.5.3-2).

Table 2.5.3-2: Description of stubble treatments after harvest of the previous crop until sowing of winter cereals.

No.	Acronym	Description
1	Repeated	Two passes of chisel ploughing (12-15 cm deep) directly after harvest and four weeks later
2	Conservation	Two passes shallow stubble tillage using a straw harrow (3-4 cm deep) with flexible tines of 16 mm diameter
3	Reduced	One pass chisel ploughing four weeks after harvest when volunteer cereals and first seedlings of *A. myosuroides* had emerged
4	Mechanical + Chemical	One pass chisel ploughing directly after harvest and one application of 4 l ha^{-1} KYLEO® (240 g L^{-1} glyphosate and 160 g L^{-1} 2,4-D, Nufarm Deutschland GmbH)) four weeks later

The second factor was the **drilling time** of winter cereals including an early date between late September and mid of October and a late date three to six weeks later from the end of October until early December (Table 2.5.3-3).

Table 2.5.3-3: Experimental details of the field experiments.

Expt.	Location	Crop (cultivar)	Year	Sowing dates	Seeding rate
1	Ihinger Hof	winter-wheat (RGT-Reform)	2018	19.10.2017 04.12.2017	300 seeds m^{-2} 350 seeds m^{-2}
2	Entringen	winter-triticale (Tulus)	2019	08.10.2018 10.11.2018	250 seeds m^{-2} 350 seeds m^{-2}
3	Ihinger Hof	winter-triticale (Tulus)	2019	25.09.2018 25.10.2018	250 seeds m^{-2} 350 seeds m^{-2}
4	Ihinger Hof	winter-wheat (Patras)	2020	08.10.2019 31.10.2019	250 seeds m^{-2} 380 seeds m^{-2}

The third factor was the **weed control method** in winter cereals including a pre-emergence application of cinmethylin (LUXINUM®) in the full recommended field rate of 0.66 l ha^{-1} (500 g a.i. ha^{-1} cinmethylin) and an untreated control. Cinmethylin was applied five days after sowing with a plot sprayer (Schachtner-Gerätetechnik, Ludwigsburg, Germany), which was calibrated for a volume of 200 l ha^{-1} and a speed of 3.6 km h^{-1}. Broadleaved weed species were controlled in all plots with synthetic auxins in spring.

2.5.3.3. Assessments

Density of *A. myosuroides* was determined 45 and 120 DAS. The first date corresponds to the end of the vegetation period in the year of drilling and the second date represents the end of tillering (vegetative growth stage). In the figures, data of the second counting are presented. *A. myosuroides* plants were counted within a 0.1 m^2 frame randomly placed four times in each plot. Grain yield was measured in a 1.5 m x 12 m strip in the center of each plot with a plot harvester (Wintersteiger, Elite 3, Ried im Innkreis, Austria). Grain weights were transformed to a homogenous water content of 14%.

2.5.3.4. Statistical analysis

For data analysis, the statistical software R (Version 3.6.2, RStudio Team, Boston, MA, USA) was used. Prior to ANOVA, the data were checked for homogeneity of variance and normal distribution of residuals. If necessary, data were square root transformed to homogenize variances and to normalize the distribution. In the figures, back transformed means are shown. In the ANOVA, stubble treatment, sowing date and herbicide treatment were included as fixed effects. Multiple mean comparison tests were performed using the Tukey HSD-Test at a significance level of $\alpha \leq 0.05$.

Combined effects of sowing date (D) and weed control (W) were calculated using the Colby function for two-way combinations (Colby, 1967), with

$$E = D + W - (D * W)/100) \qquad \text{equation 1}$$

If the expected effect (E) equals the observed effect the combined effect is additive, if less than expected it is antagonistic, if greater than expected it is synergistic. This model was originally used to calculate the combined effects of two simultaneously applied herbicides.

2.5.4. RESULTS

The factor weed control had significant effects on *A. myosuroides* density in all four experiments and on grain yield in three experiments. The factor sowing date was significant in the experiments IHO 2018, Entringen 2019 and IHO 2020. Stubble treatments did not affect *A. myosuroides* density and grain yield in the following winter cereal crop (Table 2.5.4-1)

Table 2.5.4-1: Level of significance (P-values of ANOVA) for the factors sowing date (D), stubble treatment (S) and weed control (W) in all four field experiments.

Expt.	Variable	Sowing date (D)	Stubble treatment (S)	Weed Control (W)	D x S	D x W	S x W	D x S x W
IHO 2018	ALOMY	< 0.001	n.s.	< 0.001	n.s.	n.s.	< 0.001	n.s.
	Yield	n.s.	n.s	0.011	n.s.	n.s.	0.012	n.s.
Entringen2019	ALOMY	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Yield	0.061	n.s.	< 0.001	n.s.	n.s.	0.096	n.s.
IHO2019	ALOMY	n.s.	n.s.	< 0.001	n.s.	n.s.	n.s.	n.s.
	Yield	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0444
IHO2020	ALOMY	< 0.001	n.s.	< 0.001	n.s.	n.s.	0.092	n.s.
	Yield	< 0.001	n.s.	< 0.001	n.s.	n.s.	n.s.	n.s.

Combinations of cinmethylin and delayed drilling of winter cereals provided higher *A. myosuroides* weed control efficacy than the use either weed control method alone. The total effect was additive in the experiments IHO 2018, Entringen 2019 and IHO 2019. In IHO 2020, the combined effect was synergistic, which means that delayed drilling increased cinmethylin control efficacy compared to early sowing dates (Table 2.5.4-2).

Table 2.5.4-2: Combined effects of the factors sowing date and weed control according to Colby (1967) on Alopecurus myosuroides control efficacy (WCE).

Expt.	Expected WCE (%)	Observed WCE (%)	Combined effect
IHO 2018	97	97	additive
Entringen 2019	99	99	additive
IHO 2019	98	98	additive
IHO 2020	88	91	synergistic

2.5.4.1. Effects of treatments on *Alopecurus myosuroides* density

At IHO 2018, moderate infestation rate of *A. myosuroides* density with 38 plants m^{-2} was measured in the early sown and untreated plots. Delayed drilling resulted in average *A. myosuroides* density of 11 plants m^{-2}, which corresponds to a 71% reduction. Cinmethylin controlled 97% of *A. myosuroides* plants present and emerging (Figure 2.5.4-1).

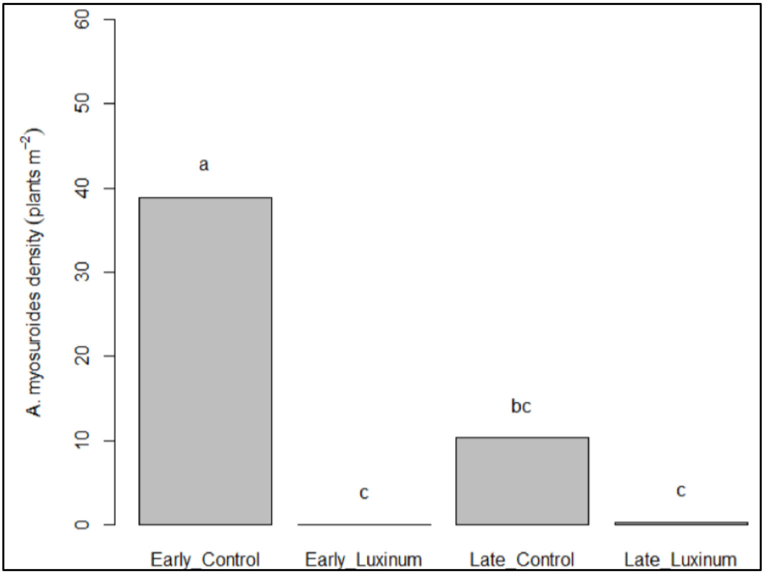

Figure 2.5.4-1: Effects of delayed autumn drilling and LUXINUM® (cinmethylin) treatment on the average Alopecurus myosuroides density at Ihinger Hof (IHO) in winter wheat 2018. Means with the same letter are not significantly different according to Tukey HSD-test at $p \leq 0.05$.

At Entringen in 2019, the effect of the three-way interaction of stubble treatment, sowing date and weed control was significant (Table 2.5.4-1). Densities in the control plots were very high and ranged from 872 to 1233 plants m^{-2} after early sowing and 464 to 996 plants m^{-2} after late sowing at the second sampling date 120 DAS. Approximately 80% of all *A. myosuroides* emerged until 45 DAS and another 20% between 45 and 120 DAT. Delaying drilling until early December reduced *A. myosuroides* density to an average of 11 plants m^{-2}. Cinmethylin application provided an additive weed control effect to late drilling. Remaining densities after any treatment sprayed with cinmethylin were less than 2 plants m^{-2}, which amounted in 98-99% *A. myosuroides* control efficacy (WCE). Cinmethylin was also effective against those approximately 20% of *A. myosuroides* plants that emerged between 45 and 120 DAS. Reduced stubble tillage resulted in slightly higher infestation rates compared to all other stubble treatments. Lowest weed densities were observed when chisel ploughing and KYLEO® (240 g L^{-1} glyphosate + 160 g L^{-1} 2,4-D, Nufarm Deutschland GmbH) applications were combined on the stubble or two passes of chisel ploughing were carried out (Table 2.5.4-3).

Table 2.5.4-3: Effects of delayed autumn drilling, stubble treatments and LUXINUM® (cinmethylin) application on average Alopecurus myosuroides density at Entringen in winter triticale 2019; 1 = repeated chisel ploughing, 2 = conservation stubble tillage, 3 = reduced stubble tillage, 4 = chisel ploughing + KYLEO®.

Sowing date	**Stubble treatment**	**LUXINUM®**	**Control**
Early	1	16 a	967 b
Early	2	16 a	1068 b
Early	3	17 a	1233 b
Early	4	8 a	872 b
Late	1	14 a	639 b
Late	2	6 a	903 b
Late	3	6 a	996 b
Late	4	3 a	464 b

In the IHO 2019 experiment, only the factor weed control showed significant effects (Table 2.5.4-1). Cinmethylin reduced average *A. mysuroides* densities from 221 to 4 plants m^{-2} (98% WCE) (Figure 2.5.4-2). From the first (45 DAS) to the second sampling date (120 DAS), average *A. myosuroides* density increased from 188 to 221 plants m^{-2} in the untreated control plots. In the treated plots, density accounted for 4 plants m^{-2} at both assessment timings indicating again a sufficient soil residual activity of cinmethylin until 120 DAS. Delayed autumn drilling slightly reduced *A. mysuroides* densities with an average of 199 plants m^{-2} compared to 241 plants m^{-2} for the early sowing date.

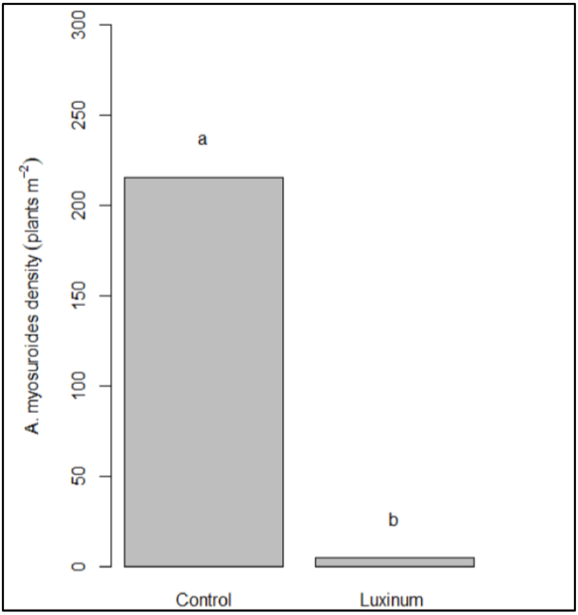

Figure 2.5.4-2: Effects of LUXINUM® (cinmethylin) treatment on the average Alopecurus myosuroides density at Ihinger Hof (IHO) in winter triticale 2019. Means with the same letter are not significantly different according to Tukey HSD-test at $p \leq 0.05$.

At IHO 2020, delayed autumn drilling and cinmethylin application significantly reduced *A. mysuroides* density (Table 2.5.4-1). Infestation rates in the early sown control plots were considerably high with an average of 282 plants m^{-2}. Delayed drilling decreased *A. mysuroides* density to 101 plants m^{-2} (64% WCE). Densities in treatments sprayed with cinmethylin were lower and amounted to 114 plants m^{-2} in the early sown plots and 24 plants m^{-2} after delayed drilling (Figure 2.5.4-3). WCE of cinmethylin increased from 58% in the early sowing date to 91% after delayed drilling. Therefore, cinmethylin application provided a synergistic effect to late drilling.

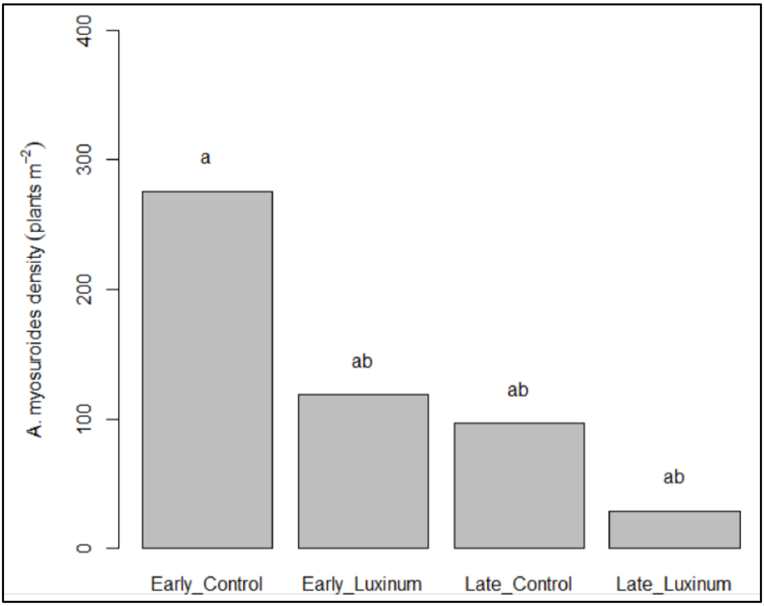

Figure 2.5.4-3: Effects of delayed autumn drilling and LUXINUM® (cinmethylin) treatment on the average Alopecurus myosuroides density at Ihinger Hof (IHO) in winter wheat 2020. Means with the same letter are not significantly different according to Tukey HSD-test at $p \leq 0.05$.

2.5.4.2. Effects of treatments on cereal grain yields

In the IHO 2018 experiment, delayed autumn drilling in combination with cinmethylin application significantly increased winter wheat yield compared to early sowing and untreated controls (Table 2.5.4-1). The cinmethylin treatment after delayed sowing resulted in a grain yield of 7.1 t ha^{-1} compared to 6.3 t ha^{-1} for the early sown treatments and 6.0 t ha^{-1} for the late drilled control plots (Figure 2.5.4-4).

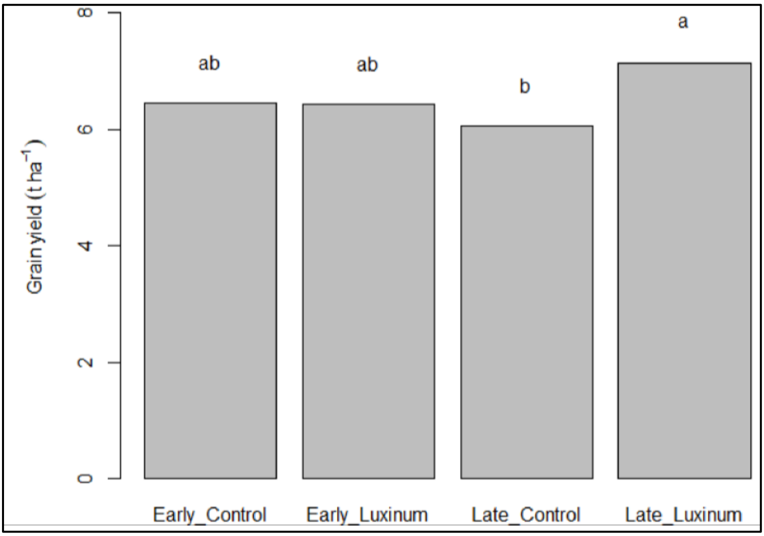

Figure 2.5.4-4: Effects of delayed autumn drilling and LUXINUM® (cinmethylin) treatment on the average winter wheat yield at Ihinger Hof (IHO) in 2018. Means with the same letter are not significantly different according to Tukey HSD-test at $p \leq 0.05$.

Grain yield in Entringen 2019 was only affected by weed control treatment (Table 2.5.4-1). The application of cinmethylin increased the average grain yield to 9.4 t ha^{-1} compared to 7.0 t ha^{-1} for the untreated control (Figure 2.5.4-5).

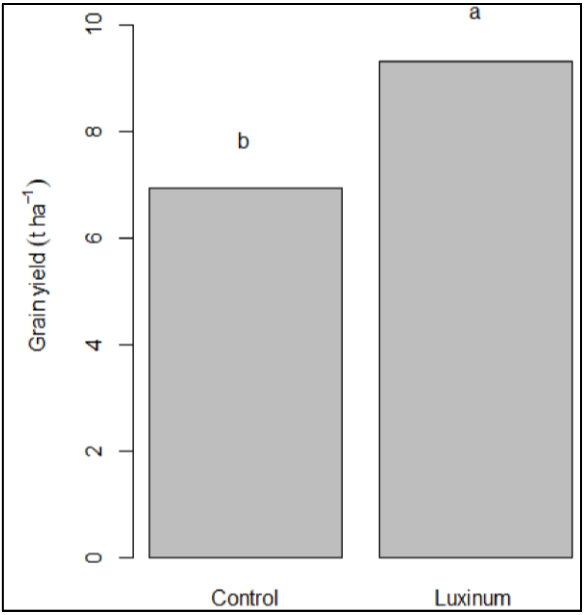

Figure 2.5.4-5: Effect of LUXINUM®(cinmethylin) treatment on the average winter triticale yield at Entringen in 2019. Means with the same letter are not significantly different according to Tukey HSD-test at $p \leq 0.05$.

At Ihinger Hof 2019, no significant effects of stubble treatment, sowing date and weed control on grain yield were observed (Table 2.5.4-1). The average grain yields amounted 5.5 t ha^{-1} to 8.5 t ha^{-1} in the control plots and 8.5 t ha^{-1} to 10.1 t ha^{-1} in the cinmethylin treatments. Yields were slightly higher after delayed sowing compared to the early sowing. Among the stubble treatments, reduced tillage resulted in slightly higher yields compared to the other treatments (Table 2.5.4-4).

Table 2.5.4-4: Effects of delayed autumn drilling, stubble treatments and LUXINUM® (cinmethylin) application on the average grain winter triticale yield at Ihinger Hof in 2019; 1 = repeated chisel ploughing, 2 = conservation stubble tillage, 3 = reduced stubble tillage, 4 = chisel ploughing + KYLEO®.

Sowing date	**Stubble tillage**	**LUXINUM®**	**Control**
Early	1	9.04 a	5.52 a
Early	2	9.15 a	5.98 a
Early	3	9.65 a	6.94 a
Early	4	9.29 a	5.95 a
Late	1	9.23 a	6.55 a
Late	2	8.52 a	7.77 a
Late	3	10.12 a	8.52 a
Late	4	9.66 a	8.36 a

Grain yields at Ihinger Hof in 2020 were influenced by sowing date and weed control (Table 2.5.4-1). Late drilling provided 2.5 t ha^{-1} higher grain yields than early sowing (Figure 2.5.4-6a) and the application of cinmethylin also increased grain yields by 2.5 t ha^{-1} compared to the untreated control (Figure 2.5.4-6b).

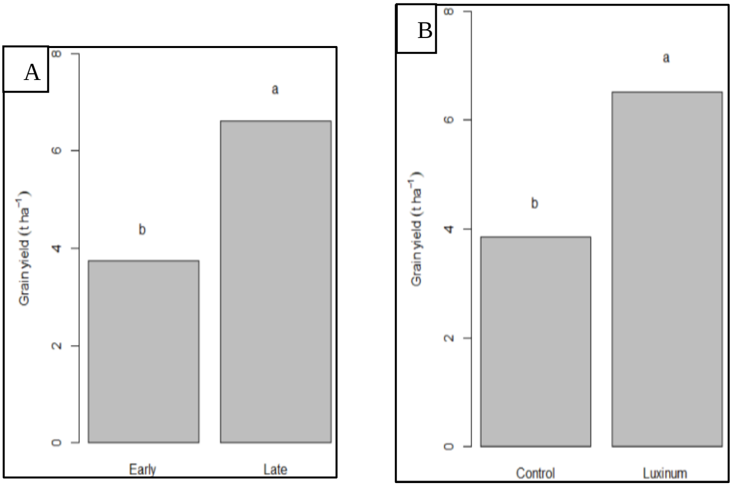

Figure 2.5.4-6: Effect of sowing date on the average winter wheat yield at Ihinger Hof in 2020 (A). Effect of LUXINUM® (cinmethylin) treatment on the average winter wheat yield at Ihinger Hof in 2020 (B). Means with the same letter are not significantly different according to Tukey HSD-test at $p \leq 0.05$.

2.5.5. DISCUSSION

The results in this study proved the first hypothesis that WCE of cinmethylin against *A. myosuroides* exceeded 80%. In average, WCE was 88% ranging from 58-99%. In a recent Australian study, cinmethylin reduced aboveground biomass of *Lolium rigidum* L. populations by 90%. Around half of the populations tested were resistant to the mitosis inhibitor trifluralin. Cinmethylin control efficacy was even slightly higher for the trifluralin resistant populations compared to the susceptible populations (Busi et al., 2020). In this Australian study, cinmethylin was similarly selective to wheat as in the present study. When the wheat seed were buried ≥1 cm in the seed bed, the emergence of wheat seedlings after pre-emergence application of cinmethylin on the soil surface was equal to the untreated control (Busi et al., 2020).

Recorded levels of weed control efficacy against *A. myosuroides* in winter cereals was similar to other pre-mergence herbicides commonly used to control *A. myosuroide*s, such as flufenacet, pendimethalin, prosulfocarb and diflufenican and combinations of those (Bailly et al., 2012; Menne et al., 2012; Menegat & Nilsson, 2019; Messelhäuser et al., 2021a). However, WCE offered by pre-emergence herbicides varied stronger between years and locations than observed after the use of post-emergence herbicides (Menegat & Nilsson, 2019; Messelhäuser et al., 2021a). Reduced WCE of pre-emergence herbicides was often observed under dry conditions on soils with high clay and organic matter content. All three factors decrease the availability of herbicides for root uptake. Residual activity of pre-emergence herbicides is often not sufficient to maintain effective weed control until the end of tillering of winter cereals, which can be as long as 150 DAS (Kudsk & Kristensen, 1992). Therefore, the use of a pre-emergence herbicide often is only one component in a more complex weed management program and needs to be combined with other weed control tactics.

The second hypothesis that delayed autumn drilling had an additive or synergistic effect on the efficacy of the pre-emergence cinmethylin application was also proven by the results of the experiments. WCE against *A. myosuroides* was always higher than 90% after the combination of late drilling and cinmethylin application few days after sowing. Combined effects were additive in 2018 and 2019 and synergistic in 2020. When herbicides are applied simultaneously, their interaction is often antagonistic, which results in lower total weed control efficacy than the sum of both single herbicides. Antagonistic effects are known for mixtures of auxins with ALS-inhibitors and combinations of auxin with glyphosate (Damalas & Eleftherohorinos, 2001) and herbicide mixtures of containing PSII-, bleachers and ALS-inhibitors (Schuster et al., 2008). This underlines the benefits of integrated weed management employing chemical and non-chemical weed control tactics. Delayed drilling of winter cereals resulted in approximately 50% lower *A. myosuroides* emergence in previous studies in the UK and Germany (Melander, 1995; Lutman et al., 2013; Menegat & Nilsson, 2019). This effect was explained with the seasonal variation of germination of *A. myosuroides*. If winter cereals were sown in late September, more *A. myosuroides* seeds will germinate after seedbed preparation and sowing (Moss, 1990; Moss, 2017). Germination rate then decreases until winter and spring and seeds will become dormant during the summer (Moss, 1990; Moss, 2017). Therefore, delayed drilling after September reduced densities of *A. myosuroides* in winter cereals (Moss, 2017). Delayed sowing of winter cereals usually does not cause any additional costs for farmers, but may increase weather related risk for cultivation and sowing on certain, especially soils with high clay or organic matter contents. In one experiment, delayed sowing favored the effect of cinmethylin, possibly because soil water content was higher in late autumn and the herbicide was better activated. In the present study, grain yields were mostly higher after late sowing than after early drilling. Therefore, delayed sowing can provide several agronomic and economic benefits to the farmers. *A. myosuroides* is a serious weed in winter cereals causing winter wheat yield losses in the UK of 5% at densities of 10-20 plants m-2, 10% at >20-35 plants m-2, 15% at >35-70 plants m-2, 20% at >70-180 plants m-2, 35% at >180-400 plants m-2 and 50% >400 plants m-2 (Blair et al., 1999). The yield benefit from cinmethylin use and late drilling amounted up to 2.5 t ha-1 of grain. This underlines the need for effective and integrated strategies to suppress *A. myosuroides* in crop rotations with high proportions of winter annual crops.

The third hypothesis that stubble treatments reduced *A. myosuroides* densities in the following winter cereal has to be rejected based on the data generated by the data. Although two passes with the chisel plough and the combination of chisel ploughing and non-selective herbicide application on the stubble in two experiments resulted in slightly lower *A. myosuroides* densities than reduced stubble tillage, all four stubble treatments tested did not result in significant different *A. myosuroides* densities in the following winter cereal crops. The uniform results of the four different stubble treatments are in contrast to previous studies reporting significant effects of stubble treatments on *A. myosuroides* densities (Moss & Clarke, 1994; Lutman et al., 2013; Schappert et al., 2018; Zeller et al., 2021). Ploughing on average reduced *A. myosuroides* densities in the following crops by 69%, stale seedbed preparation (64%), pre-emergence harrowing (52%), cover cropping (75%) and the application of glyphosate (48%) (Moss & Clarke, 1994; Lutman et al., 2013; Schappert et al., 2018; Zeller et al., 2021). Possibly, stubble treatments selected for this study had similar effects or they need to be observed over an extended period until variations of *A. myosuroides* densities can reliably be discovered. Therefore, also stubble treatments need to be considered an effective tool in integrated weed management.

2.5.6. CONCLUSIONS

The results of this study highlight the need for an integrated approach to successfully manage serious *A. myosuroides* infestations in winter cereals. Combinations of chemical and preventive methods of weed control consistently increased weed control efficacy and grain yield of winter cereals beyond chemical weed control only over all four experiments. Combining cinmethylin with delayed drilling can be considered ~~as~~ an economical and environmentally beneficial weed management approach to meet the EU-Green Deal targets. This example might give the start for exploring additional promising strategies of integrated weed control in multi-factorial experiments and on-farm research.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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2.5.7. REFERENCES

BAILLY GC, DALE RP, ARCHER SA, WRIGHT DJ & KAUNDUN SS (2012) Role of residual herbicides for the management of multiple herbicide resistance to ACCase and ALS inhibitors in a black-grass population. *Crop Protection* **34,** 96–103.

BLAIR AM, CUSSANS JW & LUTMANN PJ (1999) A biological framework for developing a weed management support system for weed control in winter wheat: weed competition and time of weed control.

BUSI R, DAYAN FE, FRANCIS I et al. (2020) Cinmethylin controls multiple herbicide-resistant Lolium rigidum and its wheat selectivity is P450-based. *Pest Management Science* **76,** 2601–2608.

CAMPE R, HOLLENBACH E, KÄMMERER L et al. (2018) A new herbicidal site of action: Cinmethylin binds to acyl-ACP thioesterase and inhibits plant fatty acid biosynthesis. *Pesticide Biochemistry and Physiology* **148,** 116–125.

COLBY SR (1967) Calculating Synergistic and Antagonistic Responses of Herbicide Combinations. *Weeds* **15,** 20.

DAMALAS CA & ELEFTHEROHORINOS IG (2001) Dicamba and atrazine antagonism on sulfonylurea herbicides used for johnsongrass (Sorghum halepense) control in corn (Zea mays). *Weed Technology* **15,** 62–67.

DAYAN FE (2003) Current Status and Future Prospects in Herbicide Discovery. *Plants (Basel, Switzerland)* **8**.

DÉLYE C, MENCHARI Y, GUILLEMIN J-P et al. (2007) Status of black grass (Alopecurus myosuroides) resistance to acetyl-coenzyme A carboxylase inhibitors in France. *Weed Research* **47,** 95–105.

DROBNY HG, SALAS M & CLAUDE J (2006) Management of metabolic resistant black-grass (Alopecurus myosuroides Huds.) populations in

Germany-challenges and opportunities. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **20,** 65.

GERHARDS R, DENTLER J, GUTJAHR C, AUBURGER S & BAHRS E (2016) An approach to investigate the costs of herbicide-resistant Alopecurus myosuroides. *Weed Research* **56,** 407–414.

HEAP I (2014) Global perspective of herbicide-resistant weeds. *Pest Management Science* **70,** 1306–1315.

KUDSK P & KRISTENSEN JL, eds (1992) Effect of environmental factors on herbicide performance. Weed Science Society of Victoria, Victoria, Australia.

LUTMAN PJW, MOSS SR, COOK S & WELHAM SJ (2013) A review of the effects of crop agronomy on the management of Alopecurus myosuroides. *Weed Research* **53,** 299–313.

MELANDER B (1995) Impact of drilling date on Apera spica-venti L. and Alopecurus myosuroides Huds, in winter cereals. *Weed Research* **35,** 157–166.

MENEGAT A & NILSSON ATS (2019) Interaction of Preventive, Cultural, and Direct Methods for Integrated Weed Management in Winter Wheat. *Agronomy* **9,** 564.

MENNE HJ & HOGREFE C (2012) Impact of multiple resistance mechanisms in black-grass (Alopecurus myosuroides Huds.) populations on the of cereal herbicides. Julius-Kühn-Archiv; 434 (1); 65-74 / Julius-Kühn-Archiv; 434 (1); 65-74.

MENNE HJ, LABER B, KERLEN D & BEFFA R (2012) Effectiveness of flufenacet in controlling resistant black-grass (Alopecurus myosuroides Huds.) – comparison of glasshouse and field trial results. Julius-Kühn-Archiv; 434 (1); 401-408 / Julius-Kühn-Archiv; 434 (1); 401-408.

MESSELHÄUSER M, SAILE M, SIEVERNICH B & GERHARDS R (2021a) Effect of cinmethylin against Alopecurus myosuroides Huds. in winter cereals. *Plant, Soil and Environment* **67,** 46–54.

MESSELHÄUSER MH, LINN AI, MATHES A, SIEVERNICH B & GERHARDS R (2021b) Development of an Agar Bioassay Sensitivity Test in Alopecurus myosuroides for the Pre-Emergence Herbicides Cinmethylin and Flufenacet. *Agronomy* **11,** 1408.

MOSS S (2017) Black-grass (Alopecurus myosuroides): Why has this Weed become such a Problem in Western Europe and what are the Solutions? *Outlooks on Pest Management* **28,** 207–212.

MOSS SR (1990) The seed cycle of Alopecurus myosuroides in winter cereals: a quantitative analysis. *Symposium on integrated weed management in cereals. Proceedings of an EWRS symposium, Helsinki, Finland, 4-6 June 1990.,* 27–35.

MOSS SR & CLARKE JH (1994) Guidelines for the prevention and control of herbicide-resistant black-grass (Alopecurus myosuroides Huds.). *Crop Protection* **13,** 230–234.

NEVE P (2007) Challenges for herbicide resistance evolution and management: 50 years after Harper. *Weed Research* **47,** 365–369.

SCHAPPERT A, MESSELHÄUSER M, SAILE M, PETEINATOS G & GERHARDS R (2018) Weed Suppressive Ability of Cover Crop Mixtures Compared to Repeated Stubble Tillage and Glyphosate Treatments. *Agriculture* **8,** 144.

SCHUSTER CL, AL-KHATIB K & DILLE JA (2008) Efficacy of Sulfonylurea Herbicides when Tank Mixed with Mesotrione. *Weed Technology* **22,** 222–230.

ZELLER A, KAISER Y & GERHARDS R (2018) Suppressing Alopecurus myosuroides Huds. in Rotations of Winter-Annual and Spring Crops. *Agriculture* **8,** 91.

ZELLER AK, ZELLER YI & GERHARDS R (2021) A long-term study of crop rotations, herbicide strategies and tillage practices: Effects on Alopecurus myosuroides Huds. Abundance and contribution margins of the cropping systems. *Crop Protection* **145,** 105613.

3. GENERAL DISCUSSION

The aim of this thesis was to evaluate different strategies of Integrated Weed Management (IWM), and examine the benefits of different strategies to control *Alopecurus myosuroides* Huds., slow down resistance development and enhance crop performance, in cereals. For this purpose, laboratory, greenhouse and field experiments were conducted. IWM implies the combination of the best practice (e.g. tillage, crop rotation) and tools (e.g. herbicide, hoe, harrow) to make cropping systems unfavourable to weeds and to minimize the impact of surviving weeds. Each individual practice should be considered as a piece of an IWM strategy (Buhler, 2002; Harker & O'Donovan, 2013). In this thesis, four articles give an overview about control strategies of *A. myosuroides* in Western European cereal cultivation. Every journal article can be read independently, and each article has already been discussed independently. In this chapter, the main results of the articles are pinpointed and discussed as a general overview of the thesis and prospects for further research are given. This chapter is structured into four main sections:

- Resistance detection for pre-emergence herbicides
- Different cultural *A. myosuroides* control strategies in winter cereals
- Direct control strategies of *A. myosuroides* in winter cereals
- Outlook of IWM strategies

The main outcomes of the laboratory; greenhouse and field experiments are discussed, and an overview of future prospects is given.

3.1. RESISTANCE DETECTION FOR PRE-EMERGENCE HERBICIDE

The repeated use of herbicides with the same mode of action, in combination with moderate use of non-chemical control measures has led to the relatively slow but steady evolution of many herbicide resistant weed populations (Dücker *et al.*, 2019). *A. myosuroides* is the most important herbicide resistant weed species infesting cereal crops in temperate regions of central Europe and was therefore chosen for this study (Moss *et al.*, 2007). Resistance affecting the efficacy of post-emergence herbicides from the ACCase (HRAC A/1) and ALS inhibitor (HRAC B/2) classes has increased in recent years (Heap, 2021). Therefore, the focus has now shifted towards pre-emergence herbicides (e.g. prosulfocarb (HRAC K3/15), flufenacet (HRAC K3/15) or pendimethalin (HRAC K1/3)), that have not yet been affected by resistance to such an extent (Bailly *et al.*, 2012).

Based on current herbicide resistance development, the farmers cultivation and weed management practices should be aimed to avoid or delay the emergence of herbicide resistance. Nevertheless, the most economical and convenient method will generally be depleted until the development of herbicide resistance forces a change in practices. Therefore, reliable and quick resistance test systems are required.

The results of Section 2.1. are based on the agar bioassay sensitivity test, which is a useful instrument for evaluating herbicide resistance to soil acting herbicides. For a method to be utilised in herbicide resistance monitoring programmes, the necessary input of time, space and material is important apart from the reliability of the test system (Moss, 1995; Rosenhauer & Petersen, 2015). Pre-emergence herbicides have particular environmental requirements regarding their efficacy. The weed control efficacy is influenced by external factors like soil moisture, soil structure, seed depth, dormancy, germination rate, and time. Their bioavailability further depends on soil properties such as pH, organic matter, and clay content (Beckie *et al.*, 2000; Colbach *et al.*, 2002a; Colbach *et al.*, 2002b). Therefore, testing of soil acting herbicides is complicated and needs specific methods and a considered interpretation of the results (Menne *et al.*, 2012a). Currently, several herbicide resistance test systems are available. The most common herbicide resistance test is the whole plant pot bioassay in the greenhouse, which provides reliable results but is time and space consuming as well as labour intensive (Beckie, 2006; Reade & Cobb, 2002; Kaundun *et al.*, 2011). Whole plant bioassay test systems, especially for pre-emergence herbicides require a large investment of resources and time.

The new agar bioassay sensitivity test is approximately twice as fast as the common whole plant pot bioassay (Beckie *et al.*, 2000). In contrast to the classic whole plant pot bioassay, the agar bioassay sensitivity test requires only about half the space. However, the operational capacity depends on the availability and size of the climatic chambers. The material cost of the agar bioassay sensitivity test system is still a factor for improvement. Since plastic containers were used once in the test version, a relatively large amount of plastic waste was produced. In the context of advancing climate change, further tests should be carried out to find an eco-friendly alternative.

Within the new agar bioassay sensitivity test external factors like light duration and soil moisture are standardized by conducting the experiments in climatic chambers with controlled environmental conditions. Furthermore, soil structure as well as seeding depth are also standardized due to the replacement of the soil substrate with an agar plate of uniform size. Accordingly, due to the absence of soil in the agar bioassay sensitivity test and the incorporation and binding of the herbicide into the agar, no sorption of the herbicide to soil particles can occur. The bioavailability of the active ingredients was therefore increased. As a result, sensitivity of the plants in the agar bioassay sensitivity test was increased compared to the whole plant pot bioassay. However, as a negative consequence, even small deviations from the required concentration of active ingredient can lead to serious deviations in the results (Beckie *et al.*, 2000; Menne & Hogrefe, 2012).

The results from the whole plant pot bioassay were reproduced for the majority (12 out of 17 populations) of populations in the agar bioassay sensitivity test. In general, the whole plant pot bioassays result in the most reliable differentiation between sensitive and resistant biotypes, although large variations within and between trials could be found because of external factors (e.g. soil moisture, temperature, light) or large variability within the populations (Menchari *et al.*, 2006; Menchari *et al.*, 2007). Considering this variability, sufficient numbers of representative plants are required to avoid inaccurate conclusions. One of the most important variables in pre-emergence testing of herbicides is the varying and usually unknown germination time of seeds, and thus the distinction between herbicide efficacy and late or non-germinated seeds. A number of factors such as seed age, seed origin, temperature, light, and moisture affect the germination time of the selected seeds (Colbach *et al.*, 2002a; Colbach *et al.*, 2002b; Colbach & Dürr, 2003). Therefore, to gain reliable assurance that the observed findings were caused by pre-emergence herbicide application, it is necessary to work with seeds that have already germinated. According to the guidlines of OECD (2006) at least 70% of the control plants have to emerge, and at least 90% of the emerged control seedlings have to survive for the duration of the study for the test to be considered valid. Although only successfully germinated seeds at the BBCH stage of 05-09 were transferred in the agar bioassay sensitivity test, not all seedlings survived and continued to grow after transfer to the experimental tray. As a result, the targeted number of plants in the agar bioassay sensitivity test could not be reliably guaranteed. Rosenhauer & Petersen (2015) encountered a very similar problem in their experiments when developing a resistance test for pre-emergence herbicides with *A. myosuroides*. However, in contrast to the experiments of Rosenhauer & Petersen (2015), over 80% of the transplanted plants in our studies survived in the untreated control.

Monitoring weed populations and rapid identification of herbicide resistance is an essential part of IWM to avoid economic losses and further spread of resistance (Burgos *et al.*, 2013).

The benefit is not only for the farmer, who saves costs by avoiding unscheduled herbicide applications, but also for the environment (Eppo, 2015).

The new agar bioassay sensitivity test is well positioned to become a useful tool for farmers. The test can be further developed to become an herbicide resistance self-test for farmers, which can be conducted by themselves on the windowsill at home. Thus, it offers the possibility to get a quick information about the resistance status of their *A. myosuroides* population and if necessary to initiate further control measures in the same season.

3.2. CULTURAL *A. MYOSUROIDES* CONTROL STRATEGIES IN WINTER CEREALS

Increased implementation of cultural control methods should allow weed populations to be reduced, based on knowledge of weed population demographics and their interaction with cropping systems. The use of these measures has the potential to lead to reduced reliance on herbicides and reduced selection for herbicide resistance (Chauvel *et al.*, 2001; Lutman *et al.*, 2013). As a result of the progress made in the development of IWM in recent years, various solutions can now be offered for the problem with persistent weeds. As part of the IWM, several cultural control measures are available for A. *myosuroides* control, including crop rotation, stubble hygiene, competitive crops, ploughing (cultivation), cover cropping, delayed autumn drilling, and in-crop cultivations (Swanton & Weise, 1991; Moss & Clarke, 1994; Chauvel *et al.*, 2001). The effectiveness of measures depends on the time of application in relation to the germination or emergence patterns of weed seeds in the soil seed bank (Chauvel *et al.*, 2001; Travlos *et al.*, 2020). In comparison to other weeds, the biological characteristics of *A. myosuroides* are relatively well known. *A. myosuroides* is characterized by a low germination base temperature (0 °C) and a short primary dormancy of 2 - 8 weeks after seed maturity (Colbach *et al.*, 2002a). The expression of primary dormancy is mainly driven by genetics and only partially dependent on environmental conditions during seed maturation on the mother plant (Baskin & Baskin, 1985; Swain *et al.*, 2006). If the environmental conditions are warm and dry during this period, the duration of primary dormancy lasts for 2 to 4 weeks. If cool and moist weather conditions dominate, primary dormancy can last up to 8 weeks (Swain *et al.*, 2006; Cook & Brooke, 2006). After termination of primary dormancy, seeds that do not germinate due to unsuitable germination conditions will enter a state of secondary dormancy, which lasts through the winter period (Moss, 1980). As temperatures rise in spring, these seeds lose their dormancy and are able to germinate (Colbach *et al.*, 2002a). This results in a wide germination period of *A. myosuroides*, ranging from late summer to early winter, only a small proportion of plants emerge in spring (Moss, 1990; Moss, 2017a). This period coincides with the sowing of winter cereals, so some seeds germinate before sowing while others germinate within the crop. Seedlings that emerge prior to seeding can be easily destroyed by tillage or the use of a non-selective herbicide, while those that emerge within the crop require the use of selective herbicides. Consequently, a primary goal of integrated weed management should be to maximize the proportion of pre-sowing seedlings that emerge. Therefore, knowledge of seed dormancy status should improve decision making regarding the timing of cultivation and seeding (Swain *et al.*, 2006).

3.2.1. STUBBLE TREATMENT

Cultural control measures before sowing of the main crop are elements of IWM, which aim to reduce the density of weeds in the following crop, thereby supporting the control level of subsequent in crop control measurements (Harker & O'Donovan, 2013). Stubble soil cultivation and/or glyphosate application, as used in this thesis, varied in their efficacy on *A. myosuroides*. Most effective and consistent was the use of cover crops (100%), double glyphosate application (61-100%), followed by ploughing (-79-52%) and false seedbed preparation (33-58%) in combination with a rotary harrow or glyphosate application. Lowest *A. myosuroides* control efficacies were achieved by the use of flat soil tillage (-175 – -14%) or deep non-turning soil tillage (- 200- -30%). Within a seven-year trial, Zeller *et al.* (2021) observed reduced infestation rates of *A. myosuroides* by inversion tillage with a mouldboard plough followed by false seedbed preparation with a harrow (shallow) and rotary harrow (deep) by up to 70% compared to reduced conservation tillage with a chisel plough alone. Corresponding results were also obtained by Lutman et al. (2013), where a change from non-inversion tillage to mouldboard ploughing reduced infestation rates of *A. myosuroides* by 69%. The effect of stubble tillage on annual weeds, e.g. *A. myosuroides* can vary considerably due to the infestation level and past cultivation history, which affect the distribution of weed seeds in the soil, the timing and frequency of tillage as well as the implement used, the soil structure and moisture, and the weather conditions both before and after cultivation (Pekrun & Claupein, 2006; Melander *et al.*, 2013; Lutman *et al.*, 2013). Also the seed production in the current year relative to the seed bank, and the dormancy status of the seeds produced are of major importance (Pekrun & Claupein, 2006; Melander *et al.*, 2013). Soil inversion changes the distribution of old and new seeds in the soil. Small-seeded seeds of *A. myosuroides* can only emerge from soil layers close to the surface (< 5 cm) (Naylor, 1970; Melander *et al.*, 2013). Through soil inversion with a mouldboard plough, seeds are moved to a deeper soil layer (> 20 cm), while non-inversion tillage (e.g., with a chisel plough) results in seed distribution in the upper soil layer (< 20 cm) (Lutman *et al.*, 2003; Gruber & Claupein, 2009). Population density of *A. myosuroides* decreases by rotational ploughing. Ploughing mainly increases mortality of weed seeds in the soil through fatal germination (Knab & Hurle, 1988). Most freshly shed seeds, which were induced to germinate, are buried to a depth from which seedlings are unlikely to emerge (Knab & Hurle, 1988). Nevertheless, annual grasses mostly have short-lived seeds, and tilling the stubble to diminish the seed bank might be counterproductive because new seeds (non-germinated) resurface after a short time. Distinct from the plough, shallow tillage or no-till techniques retain most of the freshly shed seeds in the upper 5 cm of the soil from which seedlings can easily emerge (Moss, 2017a). Tillage in general can trigger breaking of seed dormancy and initiate seed germination through light exposure, oxygenation, or mineralization (Gallagher & Cardina, 1998;

Lutman *et al.*, 2003; Swain *et al.*, 2006). Taking into advantage of this effect, the false seedbed technique can stimulate germination and emergence of weeds and volunteer crops and control plants through subsequent tillage (Travlos *et al.*, 2020). This ultimately reduces the number of weeds in the soil seed bank and number of wees in the crop (Rasmussen, 2004). However, effects of false seedbed preparation can vary highly according to prevailing weather conditions and soil moisture content appears to be the major factor determining the efficacy of a stale seedbed (Rasmussen, 2004; Travlos *et al.*, 2020). For instance, if too early and deep tillage is carried out in summer, secondary seed dormancy is induced in oil seed rape and seeds are unable to germinate, similar effects are observed in *A. myosuroides* (Pekrun *et al.*, 1998). In contrast, only few reports found a greater seed loss of freshly shed weed seeds if the seeds are left on the soil surface after crop harvest rather than being incorporated into the soil (Melander *et al.*, 2008; Jensen, 2009). In summary, control of *A. myosuroides* is improved by either retaining seeds at the soil surface and inhibiting germination or burial by ploughing (Moss, 1985; Lutman *et al.*, 2013). In case of high seed input, shallow tillage increases potential *A. myosuroides* infestation in the crop (Moss, 2017a). When tillage frequency and depth are reduced, fewer weeds are uprooted, buried or injured, resulting in either increasing reliance on other weed control methods (e.g., herbicides) or yield losses (Melander *et al.*, 2013).

3.2.2. COVER CROPPING

Cover cropping represents a different weed suppressing tactic than conventional stubble tillage, which is beneficial for IWM. Cover crops play an important role in weed suppression in the time between the crop harvest and the sowing of the next crop. Cover crops and their residues suppress weed growth due to their competition for light, water, space and nutrients, as well as the release of allelopathic substances from living or decomposing plant tissue (Farooq *et al.*, 2011; Brust *et al.*, 2014; Kunz *et al.*, 2016; Gerhards & Schappert, 2020). For sufficient weed suppression, a rapid emergence, fast soil coverage and dry matter production of cover crops are required, which is mainly determined by external factors (e.g. soil properties, field location, weather conditions) and cover crop species (Brennan & Smith, 2005). The weed control efficacy of cover crops depends on cover crop species, the amount and thickness of the mulch and the management system (sowing dates and tillage systems) as well as weather conditions during seed emergence (Creamer *et al.*, 1996; Constantin *et al.*, 2015; Blanco-Canqui *et al.*, 2015). In the present thesis we could demonstrate that well-established cover crop mixtures control 95% of all occurring weeds and even 100% of *A. myosuroides* during the fall-to-winter season averaged over two locations. The slight, but existing, difference between the type of cover crop seeding was interesting. A tendency to achieve a higher weed control efficacy by seeding the cover crops after repeated shallow tillage, than by seeding with no tillage was present. In comparison, double glyphosate application had a weed control efficacy of 93% and stubble tillage operations performed worst with a control efficacy ranging between 20-75% of all weeds. In a study of Kunz *et al.* (2016) cover crop mixtures achieved a weed control efficacy of up to 68%.

To incorporate fall-to-spring cover crops into an IWM strategy, after the harvest of the main crop, cover crops can be sown with no tillage or two weeks after repeated shallow stubble tillage. Rapid emergence of cover crops in either system is essential for successful suppression of weeds and volunteer cereals. Cover crops seeded after stubble cultivation and seedbed preparation, emerge often faster because cover crop seeds have better access to soil water (Brust *et al.*, 2014; Hartwig & Ammon, 2002). In no-till systems, direct seeders are used to ensure that cover crop seeds are incorporated into the soil and come into contact with soil water (Zimmermann *et al.*, 2011). If cover crops are sown without ploughing in an additional pass after the combine harvester, some seeds will be deposited on the straw without contact to soil moisture, which hinders germination (Brust *et al.*, 2011; Zimmermann *et al.*, 2011). Seven years after the introduction of a rye cover crop, Moonen & Barberi (2004) observed a 25% reduction in total weed seedbank density in a corn system in combination with ploughing compared to a no cover crop system. However, in the no-till corn system, subterranean clover (*Trifolium subterraneum* L.) was the most suppressive cover crop with an average reduction in weed seedbank density of 22%. The differences in weed species composition and overall seed bank density were mainly related to tillage system rather than cover crop species. Nevertheless, weed seed germination and establishment is reduced in cover crop systems, but the amount of weed seeds may increase in the upper soil layer, especially in no-till systems (Mirsky *et al.*, 2010). The combination of cover crops and different management system has the potential to decrease weed pressure, thus the actual weed control efficacy of cropping system diversification elements must always be tested in a practical context (Melander *et al.*, 2005).

In conclusion, cover crops have great potential to enhance fall-to-spring weed control in IWM strategies. The ability of cover crops to control weeds, especially problematic weeds, such as *A. myosuroides*, increases their relevance for agricultural production systems with narrow crop rotations.

3.2.3. DELAYED SEEDING

In the UK the increase in autumn sown cropping has also been associated with a trend toward ever earlier sowing. In 2017 more than 65% of the crops were seeded before the 10th of October (Cropmonitor, 2017). Early autumn seeding of winter annual crops such as winter wheat exacerbates the problem of controlling *A. myosuroides* (Moss & Clarke, 1994; Melander, 1995; Lutman *et al.*, 2013; Moss, 2017a). *A. myosuroides* is so closely associated with winter cereal growing, replacing early autumn-sown crops with late seeded crops or even better with spring sown crops reduces infestation levels to a high extend. However, delayed autumn drilling must always be weighed against the risk of reduced yield (Amann *et al.*, 1992; Melander, 1995; Rasmussen, 2004). Seeding of winter cereals after the mid of October, resulted in significantly lower *A. myosuroides* populations in years with extended primary dormancy. Late seeding followed by dry summers may result in poor establishment of the crop and low crop yields (Orson, 1996). In general, the later the sowing date, the greater the yield reductions. Studies in Denmark have shown that wheat yields decrease on average by 6% when the sowing date is shifted from September 20 by 40 days to October 30 (Melander, 1995). Similar results could be observed in the conducted studies. The efficacy of delayed autumn drilling on *A. myosuroides* can vary highly between -64% and 97% (Lutman *et al.*, 2013; Menegat & Nilsson, 2019). In the conducted experiments described in the present thesis, about 50% reduction of *A. myosuroides* was achieved by delayed autumn drilling until mid/end of October. It is likely that the effects from delayed seeding are later influenced by weather conditions during seed maturation in early summer and *A. myosuroides* emergence in fall (Swain *et al.*, 2006; Lutman *et al.*, 2013; Menegat & Nilsson, 2019). Therefore, it is concluded that delayed seeding has its greatest benefit during wet fall months, which promote early germination of *A. myosuroides* seeds after harvest (July-September). In dry autumns, most seedlings do not germinate before drilling and seedlings are more likely to emerge after drilling, which in Germany is typically in September or October (Lutman *et al.*, 2013). This conclusion can be supported by a study of Colbach *et al.* (2005) which predicts that delaying seeding until mid-October will only effectively reduce *A. myosuroides* populations if there was adequate precipitation in September.

3.2.4. FURTHER CULTURAL CONTROL METHODS

Crop rotations can be very effective for controlling weeds in IWM strategies (Swanton & Murphy, 1996). Crop diversification encourages operational diversity that in turn can facilitate improved weed management. Different crops are seeded and harvested at different times of the year. If sufficient differences exist in germination requirements of crops and weeds then seed date can be manipulated to benefit the crop (Blackshaw *et al.*, 2008). One of the most problematic weed species in western European countries is *A. myosuroides*, which is particularly favoured by rotations dominated by autumn-sown crops. Rotating winter- and summer-annual crops effectively suppresses weed species that predominantly germinate in spring or autumn such as *A. myosuroides* (Lutman *et al.*, 2013). Within several studies a reduction of *A. myosuroides* density through crop rotation could be determined. While Lutman *et al.* (2013) achieved a mean reduction of 88% by implementing spring barley in the crop rotation, Zeller *et al.* (2018) obtained a 50% reduction by implementing 50% spring barley and maize in the crop rotation. Even cultivar choice has an effect on weed suppression. A reduction of 30% of *A. myosuroides* heads m^{-2} could be achieved by comparing the most competitive winter wheat cultivar with the least competitive (Lutman *et al.*, 2013). There are several reasons why some wheat cultivars are more competitive than others: taller plants, more planophile leaves, greater tillering, higher growth rate as well as allelopathy are some of them (Seavers & Wright, 1999; Hoad *et al.*, 2008; Zerner *et al.*, 2008; Bertholdsson, 2011). However, substantial densities of *A. myosuroides* may remain in the field. Therefore, to achieve a sufficient control of 95%, it is necessary to include further measures (Moss, 2017a).

3.3. DIRECT CONTROL STRATEGIES OF *A. MYOSUROIDES* IN WINTER CEREALS

Grass-weeds like *A. myosuroides* became more abundant in Europe mainly due to high percentages of autumn sown crops in cropping systems and reduced tillage practices combined with continuous applications of herbicides with the same mode of action (Gerhards *et al.*, 2020). As a result, the excessive use of herbicides, combined with limited use of non-chemical control methods, has led to widespread resistance, in particular to ACCase and ALS-inhibiting post-emergence herbicides (Heap, 2014c). With the declining performance of post-emergence herbicides, farmers need to use more pre-emergence herbicides, which tend to be less susceptible to resistance, and to focus more on non-chemical control methods, which also include hoeing and harrowing (Melander *et al.*, 2005; Moss, 2017a).

3.3.1. CHEMICAL CONTROL

''The destruction of weeds by chemicals must of course be supplementary to crop rotation, summer fallowing and other control methods, which will always have a prominent place.'' (National Research Council, 1929).

In recent years, the use of pre-emergence herbicides (e.g., flufenacet (HRAC K3/15), pendimethalin (HRAC K1/3), prosulfuocarb (HRAC K1/3), diflufenican (HRAC F1/12)) has increased significantly due to the progressive development of herbicide resistances. Most of the pre-emergence herbicides used to control *A. myosuroides* belong to a different mode of action than post-emergence herbicides. Because pre-emergence herbicides have not been used as frequently as post-emergence herbicides at the moment, these classes are currently less affected by resistance. (Menne & Hogrefe, 2012). This statement could be supported by our experiments, whereby flufenacet achieved an average control efficacy of 75%. In previous studies, the efficacy of flufenacet varied in terms of control efficacy targeting *A. myosuroides*. Bailly *et al.* (2012) achieved a control efficacy of up to 98% whereas Menne & Hogrefe (2012) achieved a control efficacy of 30-60%. Nevertheless, resistance against pre-emergence herbicides is also increasing and new modes of action are needed. The relatively old substance cinmethylin was rediscovered as a pre-emergence herbicide in winter cereals to control *A. myosuroides*. Within this thesis, densities of *A. myosuroides* varied between 40–900 plants m^2. Cinmethylin controlled more than 90% of *A. myosuroides* plants when averaged over five field experiments. Comparing two different rates of cinmethylin, even a reduced rate of 375 g a.i. ha^{-1} was able to reduce *A. myosuroides* density by 86-97% and the full rate of 495 g a.i. ha^{-1} controlled 95-100%.

The variation in control efficacy of pre-emergence herbicides depends mainly on soil moisture conditions, temperature and plant growth stage at the time of application, other factors are herbicide dose, persistence, spraying accuracy, seedbed conditions, weed emergence patterns and crop competition (Moss & Hull, 2009).

To maximize efficacy, pre-emergence herbicides require sufficient precipitation or irrigation within the first two weeks after application to ensure dissolving of the herbicide in the soil water solution and thus uptake by emerging weeds (Buhler, 1991). In general, flufenacet was less effective than cinmethylin in years with low precipitation. Variations in herbicide efficacy might be enhanced by insufficient incorporation into the soil within this period, which generally reduces the bioavailability and efficacy of the herbicide (Stewart *et al.*, 2012). In addition to dry soil conditions also higher temperatures result in enhanced growing of the plants and therefore influence the efficacy of pre-emergence herbicides (Menne & Hogrefe, 2012). In particular, soil temperature affects the efficacy of pre-emergence herbicides. The efficacy of pre-emergence herbicides in general decreases as soil temperature increases, mainly due to increased volatilization, herbicide metabolism in plants, and/or photochemical and microbial degradation in the soil (Prueger *et al.*, 2017; Zimdahl & Clark, 1982). Furthermore, increasing soil temperatures stimulate the germination and growth of many weeds, thus reducing the period of time in which weeds can be affected by pre-emergence herbicide (Varanasi *et al.*, 2016b).

The preferred germination period of *A. myosuroids* is autumn, although late emergence is also possible in spring. For this reason and to reduce the risk of resistance development the use of herbicide mixtures and sequences with different modes of actions are promoted. However, a practical advantage of a sequence of two herbicides compared to a mixture may be that a better assessment of the efficacy of each herbicide is possible if sufficient time elapses between applications. A possible disadvantage of sequences is that two separate applications are necessary, therefore it is possible that the later application will result in a lower efficacy because the weeds are larger. However, in cases where the first application provides adequate control, the second may be unnecessary (Moss *et al.*, 2007). Especially, herbicide sequences that include a residual partner are important to control late germination, especially in resistance situations (Bailly *et al.*, 2012). In our study the combination of the two pre-emergence herbicides cinmethylin and flufenacet achieved an average control efficacy of 60% and the sequence of application of pre-emergence herbicide cinmethylin or flufenacet followed by a post emergence compound resulted in average control efficacies of 80-90%. Similar results were achieved by Bailly *et al.* (2012) whereby a single application of prosulfocarb and diflufenican as a pre-emergence treatment achieved only 70% control efficacy, while a sequence with post emergence herbicide pinoxaden and pendimethalin controlled up to 100%. The pre-emergence treatment had reduced the weed cover and weakened the seedlings enough to enable good control with the post-emergence herbicide application.

However, to reduce the pressure on chemical compounds these measures can be complemented with cultural and preventive measures. Within the conducted studies, late sowing (mid/end October) in combination with cinmethylin achieved control levels of more than 75%. With delayed sowing, soil and air temperature during and after herbicide application are lower, this probably resulted in a reduced growth rate of *A. myosuroides* and hence better uptake of the herbicide through the mesocotoyl (Menegat & Nilsson, 2019).
Within this thesis, shallow tillage by disc and straw harrow or false seedbed preparation followed by 495 g ai ha^{-1} cinmethylin were able to achieve consistent control efficacies of 97-100% in both experimental years. Furthermore, the combination of no-tillage followed by a cinmethylin application achieved control efficacies of 86-100 %. Tillage has a major impact on seed germination and seedling emergence, appearing to alter the dynamics of weed emergence and thus the efficacy of pre-emergence herbicides (Concenço *et al.*, 2011). In contrast to our studies, Chauhan *et al.* (2006) suggested that herbicide applications tended to be less effective against grass weeds in systems with non-inversion tillage than with inversion tillage. Regarding residual herbicides, this may be due to (1) increased adsorption in surface layers of non-tilled soil due to higher organic matter content; (2) physical interception and adsorption of crop residues; (3) and the development of herbicide resistance that intercepts the applied herbicide (Erbach & Lovely, 1975; Chauhan *et al.*, 2006). Nevertheless, in some years of our experiments herbicide efficacy under non-inversion tillage tended to be less effective against *A. myosuroides*. This may be due to later germination in autumn, when herbicide efficacy has declined, and this may also contribute to the lower performance of pre-emergence herbicides in general.
Improved knowledge and understanding of emergence patterns of grasses in autumn sown crops in different tillage regimes could improve the timing of

direct control measures, whether chemical or non-chemical, which could optimize control efficacy and limit herbicide use (Scherner *et al.*, 2017).

3.3.2. MECHANICAL WEED CONTROL

Concerns about negative site-effects (residues in food, resistant weed species) of herbicides, are the main reason for increasing interest in mechanical weed control methods such as harrowing or hoeing. The feasibility and effectiveness of these alternative methods highly depends on the crop growth stage, the present weed species composition, soil and weather conditions (Kurstjens & Kropff, 2001; van der WEIDE *et al.*, 2008; Rasmussen *et al.*, 2009). The experience and knowledge of the farmer have to be considered, too. Under optimal weather and field conditions, mechanical weed control can be as effective as single herbicide applications against broadleaf weeds (Home *et al.*, 2002; Wiltshire *et al.*, 2003; Spaeth *et al.*, 2020).

Among mechanical weed control methods, weed harrowing in cereals is one of the most promising methods because of its labour efficiency (Rasmussen, 1992). The working mechanism of harrowing implies whole field cultivation and therefore includes risk of crop damage. The weed control mechanism of harrowing is mainly due to soil burial, but also uprooting plays a role when weeds are small (Rydberg, 1994; Kurstjens & Kropff, 2001). The intensity of harrowing can be regulated by modifying the driving speed, the number of consecutive passes and the tine angle (Rydberg, 1994; Rasmussen & Svenningsen, 1995). The challenge is to achieve a high degree of weed control while keeping crop damage as low as possible (high selectivity) (Rasmussen *et al.*, 2008; Gerhards *et al.*, 2021). In cereals, there are two time periods for harrowing, pre-emergence before plant emergence and post-emergence after plant emergence (Rasmussen & Svenningsen, 1995; Melander *et al.*, 2005). Brandsaeter *et al.* (2012) showed a weed control efficacy (WCE) of 26% after pre-emergence harrowing and 47% after post-emergence harrowing in cereals. They achieved the best weed control efficacy when pre- and post-emergent weed harrowing were combined (61%). But, harrowing can also achieve up to 80–90% WCE against mostly annual broad-leaved weeds in spring cereals like spring barley, spring oats and triticale, when weather conditions are dry, sunny and the field has a fine seedbed (van der WEIDE *et al.*, 2008; Rasmussen *et al.*, 2009). However, due to its moderate efficacy against grasses and perennial weeds, harrowing should be combined with other preventive (crop rotation, tillage practices, cover cropping) and curative (hoeing, chemical weed control) tactics of weed control (Melander *et al.*, 2005; Hillocks, 2012).

As a second mechanical weed control or in addition to harrowing, a hoe can be used in cereals. Hoeing is able to control larger weeds and annual grasses that are difficult to control with flexible tine harrows (Melander *et al.*, 2012). The weed control mechanism of a hoe is cutting and burying weeds. Furthermore, the risk of crop damage is lower with hoeing than with harrowing because of inter-row treating (Lötjönen & Mikkola, 2000). Additionally, the hoe can be equipped with intra-row tools such as finger weeders. The efficiency of weed control with a hoe is less affected by the timing of treatment, soil moisture and soil type than with a harrow, because the weeds are cut by the hoe shares (Machleb *et al.*, 2020). Machleb *et al.* (2018) were able to achieve control efficiencies of 89% using an inter-row hoe with no-till sweeps and goosefoot blades in spring barley.

3.4. OUTLOOK OF IWM STRATEGIES

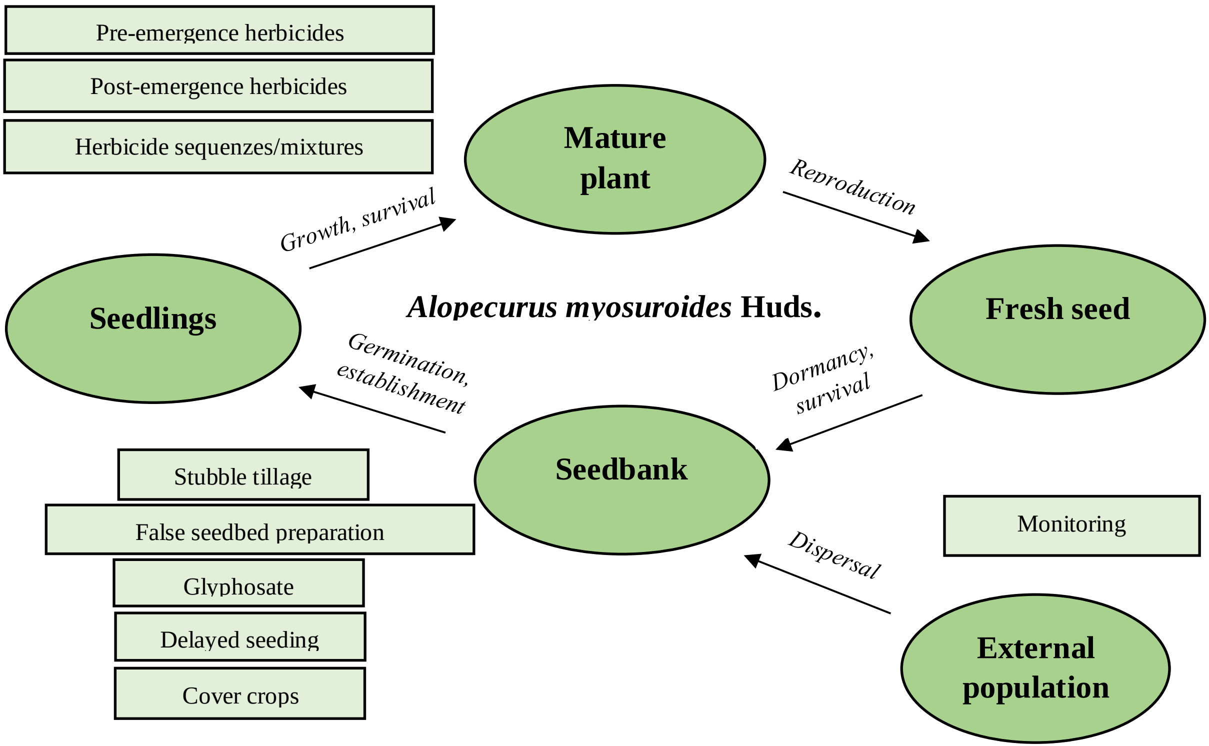
IWM is defined as the combination of several weed management strategies (biological, chemical, cultural, or physical) before, during and after the life cycle of a crop. It might be concluded, incorrectly, that IWM implies that herbicides should be avoided by favouring alternative weed management strategies. All weed management strategies that are constantly repeated exerts strong selection pressure on weeds. Therefore, on long-term, weeds will resist any frequently repeated weed control strategies. In an IWM strategy, using a variety of weed control methods are more important than trying to eliminate a single method (Harker & O'Donovan, 2013).

The implementation of an herbicide with an active ingredient that has a new mode of action offers the opportunity to correct mistakes previously made by the repeated application of herbicides with the same mode of action. The use of a new active ingredient such as cinmethylin, as part of a strategy rather than as an exclusive control strategy can slow down or, in the best case, prevent the development of resistance to that active ingredient. Scientists recommend multiple herbicide application methods or recommend the use of more than one herbicidal mode of action. These methods are important and also part of IWM, known as integrated herbicide management. However, this strategy should be combined with other biological, physical and cultural management strategies to ensure long-term and sustainable control of *A. myosuroides*.

Considering results obtained, cultural strategies such as delayed sowing and stubble cultivation are already providing control efficiencies. Nevertheless, the combination of inter-row hoeing and in-row herbicide application should also be considered for future control strategies in cereals. Also, a site-specific application of herbicides can minimize selection pressure and conserve resources.

4. SUMMARY

Alopecurus myosuroides Huds. is one of the most problematic grass weeds in cereal production in Western Europe. This grass weed spread rapidly due to the repeated and intensive use of herbicides with the same mode of action and changes in arable cropping and tillage systems. The increased cultivation of winter annual crops, reduced tillage and early sowing dates of cereals lead to increasing population densities of *A. myosuroides*. Herbicide applications are the common agricultural practice for successful control of *A. myosuroides* due to its high flexibility and low cost. However, due to European and national restrictions and the growth of herbicide-resistant populations, farmers are forced to reduce herbicide use to minimize chemical impacts on the environment and food chain. To reduce herbicide use in agriculture while still maintaining high yields and adequate weed control, a diverse weed control strategy is required. As a holistic approach for reducing herbicide use, integrated weed management (IWM) is a diversification of the control strategy of *A. myosuroides*. The essential parts in composing a successful IWM system to control *A. myosuroides* are:


Pre-emergence herbicides
Post-emergence herbicides
Herbicide sequenzes/mixtures
Mature plant
Reproduction
Growth, survival
Seedlings
Alopecurus myosuroides Huds.
Fresh seed
Germination, establishment
Dormancy, survival
Seedbank
Stubble tillage
False seedbed preparation
Glyphosate
Delayed seeding
Cover crops
Dispersal
Monitoring
External population

In this thesis, all the named aspects of IWM were examined and combined to test for a successful *A. myosuroides* control strategy in winter cereals. Special attention was paid to cinmethylin, a pre-emergence herbicide with a new mode of action in winter cereals to control *A. myosuroides*.

The first article comprised the development of an agar bioassay sensitivity test to determine sensitivity differences in *A. myosuroides* populations to pre-emergence herbicides containing flufenacet and the re-discovered substance cinmethylin. Thus, 18 *A. myosuroides* populations were tested for sensitivity twice by the new agar bioassay sensitivity test and the whole plant pot bioassay in greenhouse. All of the tested populations did not show reduced sensitivity to cinmethylin, but differences in resistance factors were observed between the agar bioassay sensitivity test and the standard whole plant pot bioassay in the greenhouse. Nevertheless, it was possible for the most part to confirm the results for cinmethylin and flufenacet of the standardized greenhouse whole plant pot bioassay in the agar bioassay sensitivity tests and hence create a reliable, faster test system. Due to the fact that cinmethylin is not yet marketed in Europe, these resistance factors can also be seen as a baseline sensitivity for *A. myosuroides*. and later used for monitoring changes in weed populations.

The second article focused on cultural measures like cover crop mixtures, various stubble tillage methods and glyphosate treatments and their effect on total weed infestation in particular on *A. mysouroides* and volunteer wheat. Within two field experiments, the cover crop mixtures and the dual glyphosate application achieved a control efficacy of *A. myosuroides* of up to 100%, whereas stubble tillage and the single glyphosate treatment did not reduce *A. myosuroides* population significantly. The results demonstrated, that besides a double glyphosate application, well developed cover crop mixtures have a great ability for weed control, even for *A. myosuroides*.

The third article also dealt with the combination of cultural measures (delayed seeding) and herbicide application and their influence on *A. myosuroides* control efficacy and yield response of winter wheat and triticale. Over a period of three years, five field experiments were set up to show the potential of the pre-emergence herbicide cinmethylin and other common pre – emergence herbicides as well as sequences and mixtures of herbicides. Within four of the experiments, the effect of early and late seeding of winter cereals was included as second factor. Results indicate that cultural methods such as delayed seeding can reduce *A. myosuroides* populations up to 75%, although to achieve control efficacy of > 95%, supplementary herbicides should be used. The control efficacy of the herbicides on *A. myosuroides* varied between the years and their respective weather conditions, between 43-100%. The combination of late seeding and the use of cinmethylin offers the possibility of high control efficacy of *A. myosuroides* compared to a single application.

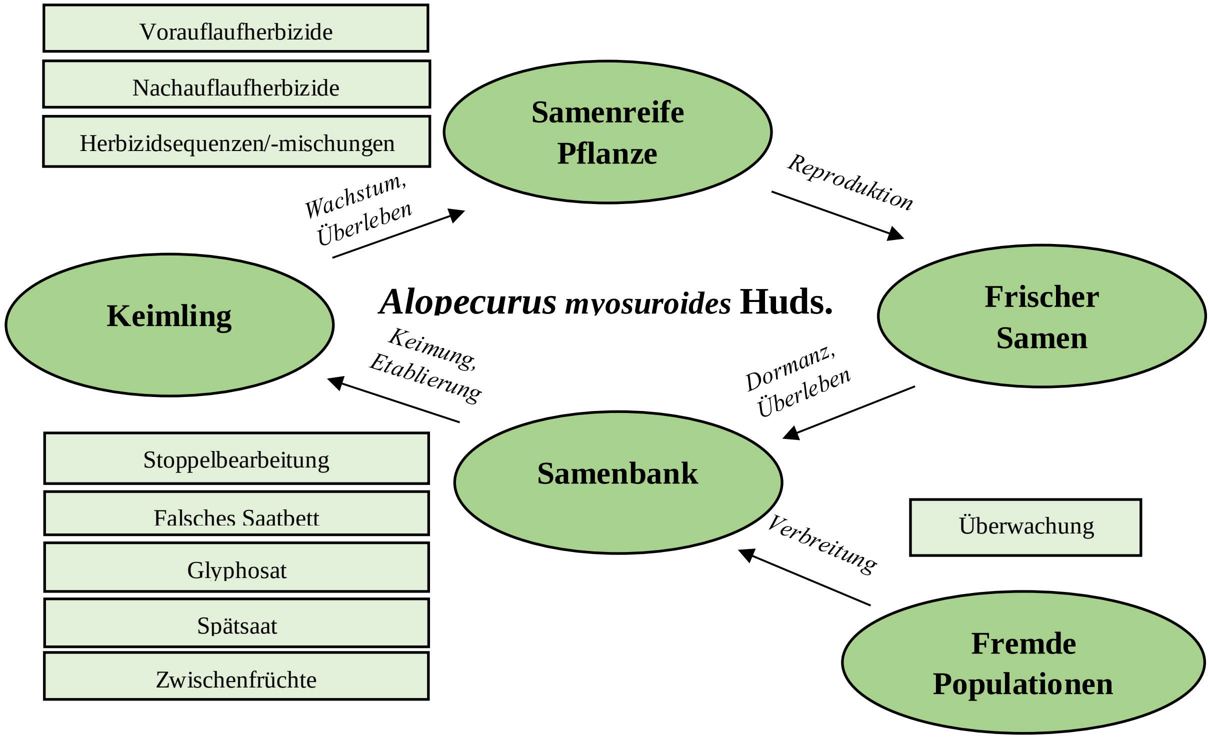
In the fourth article, a two-year experiment on two experimental sites was set up with a special focus on stubble tillage methods, glyphosate application and the application of the pre-emergence herbicide cinmethylin in two rates. Different stubble tillage techniques and combinations with cinmethylin varied in their efficacy between trials and years. Control efficiencies of 99-100% were achieved by ploughing, double glyphosate application or via false seedbed preparation, each in combination with a cinmethylin application. Even though stubble tillage were not consistent in their efficacy contribution to a cinmethylin treatment, they were able to provide similar results to a sequence application in which cinmethylin followed a double application of glyphosate.

In the last articel, over a period of three years the new pre-emergence herbicide cinmethylin was tested in combination with stubble treatments and delayed drilling of winter annual cereals in four field experiments in winter wheat and winter triticale in Southwestern Germany. Cinmethylin controlled 58-99% of *A. myosuroides* plants until 120 days after sowing. Additive and synergistic effects of cinmethylin and delayed drilling were found for all studies. Stubble did not result in significant different *A. myosuroides* densities in the following winter cereal crops. Winter wheat and winter triticale grain yields were significantly increased by the use cinmethylin combined with delayed drilling.

Considering all cultural practices, outcomes were highly variable and effects inconsistent. The diversity of weed communities for weed control is addressed by combining different management techniques. In this study, the focus was set on monitoring, cultural and direct weed control methods. Specifically, cultural measures prior to seeding of the main crop created unfavourable growing conditions for weeds. Direct control measures such as various herbicide applications can be used as a stand-alone or supplemental method of weed control later in the growing season. Considering especially *A. myosuroides*, a diverse control strategy needs to be implemented to ensure a sustainable and reduced herbicide use, high control levels, minimized crop damage, safeguarded grain yields and reduced risk of resistance development. However, IWM measures imply increased system complexity, which may make their adoption by farmers difficult. Nevertheless, the results show that cinmethylin can be successfully used for weed control systems in combination with different stubble tillage methods, glyphosate application, delayed seeding, or herbicide sequences and mixtures, making it a valuable tool in integrated weed and resistance management strategies with its novel and unique mode of action.

5. ZUSAMMENFASSUNG

Alopecurus myosuroides Huds. ist eines der problematischsten Grasunkräuter im Getreideanbau in Westeuropa. Der verstärkte Anbau von einjährigen Winterkulturen, der wiederholte Einsatz von Herbiziden mit gleichen Wirkmechanismen, reduzierte Bodenbearbeitung und frühe Aussaattermine von Getreide führen zu steigenden Populationsdichten von *A. myosuroides*. In der landwirtschaftlichen Praxis wird häufig aufgrund ihrer hohen Flexibilität, Bekämpfungserfolge sowie den geringen Kosten auf Herbiziden zur erfolgreichen Bekämpfung von *A. myosuroides* zurückgegriffen. Durch die Zunahme an Herbizidresistenten Unkrautpopulationen, sowie durch europäische und nationale Beschränkungen sind die Landwirte gezwungen, den Herbizideinsatz zu reduzieren, um die negativen Auswirkungen auf die Umwelt und die Rückstände in Nahrungskette zu minimieren. Um diese Ziele zu erfüllen und gleichzeitig hohe Erträge und eine angemessene Unkrautbekämpfung zu gewährleisten, ist eine vielfältige Unkrautbekämpfungsstrategie erforderlich. Als ganzheitlicher Ansatz zur Reduzierung des Herbizideinsatzes und die damit verbundenen negativ Auswirkungen, stellt das integrierte Unkrautmanagement (IWM) eine Diversifizierung der Bekämpfungsstrategie von *A. myosuroides* dar. Die wesentlichen Bestandteile eines erfolgreichen IWM-Systems zur Bekämpfung von *A. myosuroides* sind:


Vorauflaufherbizide
Nachauflaufherbizide
Herbizidsequenzen/-mischungen
Samenreife Pflanze
Reproduktion
Wachstum, Überleben
Keimling
Alopecurus myosuroides Huds.
Frischer Samen
Keimung, Etablierung
Dormanz, Überleben
Stoppelbearbeitung
Samenbank
Falsches Saatbett
Glyphosat
Spätsaat
Zwischenfrüchte
Verbreitung
Überwachung
Fremde Populationen

Um eine integrierte Bekämpfungsstrategie von *A. myosuroides* in Wintergetreide zu entwickeln, wurden im Rahmen dieser Arbeit alle oben genannten Aspekte von IWM einzeln untersucht bzw. kombiniert. Besondere Aufmerksamkeit wurde hierbei dem neuen Wirkstoff Cinmethylin geschenkt, welches durch seinen neuen Wirkungsmechanismus eine Erweiterung des Vorauflaufherbizidrepertoires in Wintergetreide zur Bekämpfung von *A. myosuroides* darstellt. Die Forschungsziele wurden im Rahmen von vier wissenschaftlichen Publikationen behandelt.

Der erste Artikel befasst sich mit der Entwicklung eines Agar Biotests zur Bestimmung von Sensitivitätsunterschieden in *A. myosuroides* Populationen gegenüber den in Vorauflaufherbiziden enthaltenen Wirkstoffen Flufenacet und Cinmethylin. Hierzu wurden 18 *A. myosuroides* Populationen in jeweils zwei Durchläufen sowohl durch das neue Agar Testsystem als auch durch den herkömmlichen Gewächshaus Test hinsichtlich ihrer Sensitivität getestet. Alle getesteten Populationen zeigten keine reduzierte Sensitivität gegenüber Cinmethylin, dennoch wurden Unterschiede in den Resistenzfaktoren zwischen dem Agar Biotest und dem Standard Gewächshaus Biotest festgestellt. Die Ergebnisse des Gewächshaus Biotests konnten zum größten Teil für Cinmethylin und Flufenacet durch den Agar Biotest reproduziert werden und damit ein effizienteres Testsystem geschaffen werden. Da Cinmethylin in Europa noch nicht kommerziell verfügbar ist, können diese Resistenzfaktoren auch als Basis-Sensitivität für *A. myosuroides* angesehen und später zur Überwachung von Veränderungen in Unkrautpopulationen verwendet werden.

Der zweite Artikel konzentrierte sich auf vorbeugende Unkrautbekämpfungsmaßnahmen wie Zwischenfruchtmischungen, verschiedene Stoppelbearbeitungsmethoden und Glyphosatbehandlungen und deren Auswirkung auf die Unkrautbekämpfung, insbesondere auf *A. myosuroides* und Ausfallweizen. In zwei Feldversuchen auf zwei Standorten konnten die Zwischenfruchtmischungen sowie die doppelte Glyphosatanwendung einen Bekämpfungserfolg von *A. myosuroides* von bis zu 100% erreichen. Die Ergebnisse zeigen, dass neben einer doppelten Glyphosatanwendung auch gut etablierte Zwischenfruchtbestände das Potential für einen hohen Unkrautbekämpfungserfolg von *A. myosuroides* bieten.

Der dritte Artikel befasste sich mit der Kombination vorbeugender Unkrautbekämpfungsmaßnahmen (Früher-, Später-Saattermin) sowie dem Einsatz unterschiedlicher Herbizide. Hierbei wurde deren Einfluss auf den Bekämpfungserfolg von *A. myosuroides* sowie auf den Ertrag von Winterweizen und Triticale untersucht. Über einen Zeitraum von drei Jahren wurde auf zwei Standorten fünf Feldversuche angelegt, um das Potenzial des Wirkstoffes Cinmethylin sowie anderen zur Bekämpfung von *A. myosuroides* verwendeten Wirkstoffen sowie Herbizidsequenzen/-mischungen aufzuzeigen. In vier der Versuche wurde der Effekt von früher und später Aussaat von Wintergetreide als zweiter Faktor einbezogen. Die späte Aussaat von Wintergetreide reduzierte die Dichte von *A. myosuroides* in drei von vier Versuchen im Vergleich zur früh gesäten unbehandelten Kontrolle. Die Ergebnisse zeigen, dass vorbeugende Bekämpfungsmaßnahmen wie die Spätsaat, die Bestandsdichte von *A. myosuroides* um bis zu 75% reduzieren konnte. Der durch herkömmliche Herbizide erreichte Bekämpfungserfolg von *A. myosuroides* variierte zwischen den Jahren und den jeweiligen Witterungsbedingungen zwischen 43-100%. Es gab jedoch keine signifikanten Unterschiede über alle Behandlungen hinweg. Dennoch sollten zum Erreichen eines Bekämpfungserfolges von > 95% zusätzliche zur Wahl des Saattermines, Herbizide eingesetzt werden. Abgeleitet aus den Ergebnissen, bietet die Kombination aus einer Spätsaat und dem Einsatz von Cinmethylin im Vergleich zu einer Einzelanwendung die Möglichkeit hohe Bekämpfungserfolge von *A. myosuroides* zu erzielen.

Im vierten Artikel wurde über eine Periode von zwei Jahren auf zwei Standorten vier Feldversuche angelegt, bei dem die Kombination aus Stoppelbearbeitung, einer Glyphosatanwendung sowie die Anwendung des in Vorauflaufherbizid enthaltenen Wirkstoffs Cinmethylin in zwei Aufwandmengen im Vordergrund standen. Durch die Kombinationen von Cinmethylin mit pflügen, doppelter Glyphosatanwendung oder durch die Kombination mit einer falschen Saatbettbereitung konnten Bekämpfungserfolge von *A. myosuroides* von 99-100% erzielt werden. Betrachtet man alle durchgeführten vorbeugenden Unkrautbekämpfungsmaßnahmen ergab sich eine hohe Variabilität der Bekämpfungserfolge.
Im letzten Artikel wurde über einen Zeitraum von drei Jahren das neue Vorauflaufherbizid Cinmethylin in Kombination mit verschiedenen Stoppelbearbeitungsstrategien und einer verzögertern Aussaat von Wintergetreide in vier Feldversuchen mit Winterweizen und Wintertriticale im Südwesten Deutschlands getestet. Cinmethylin kontrollierte 120 Tage nach der Aussaat, 58-99 % der *A. myosuroides*-Pflanzen. Additive und synergistische Effekte von Cinmethylin und verzögerter Aussaat wurden in allen Studien festgestellt. Die verschiedenen Stoppelbearbeitungsstrategien führten zu keinen signifikant unterschiedlichen *A. myosuroides*-Dichten im Wintergetreide. Die Kornerträge von Winterweizen und Wintertriticale wurden durch den Einsatz von Cinmethylin in Kombination mit einer verzögerten Aussaat signifikant erhöht.
In dieser Studie lag der Fokus auf dem Monitoring, vorbeugenden sowie chemischen Unkrautbekämpfungsmethoden. Konkret bedeutet dies, dass durch vorbeugende Bekämpfungsmaßnahmen vor der Aussaat der Hauptkultur ungünstige Wachstumsbedingungen für Unkräuter geschaffen wurden. Direkte Bekämpfungsmaßnahmen wie verschiedene Herbizidanwendungen können als alleinige oder ergänzende Methode der

Unkrautbekämpfung später in der Vegetationsperiode eingesetzt werden. Insbesondere im Fall von *A. myosuroides* muss eine vielfältige Bekämpfungsstrategie umgesetzt werden, um einen nachhaltigen und reduzierten Herbizideinsatz, hohe Bekämpfungserfolge, reduzierte Ernteschäden, gesicherte Kornerträge und ein reduziertes Risiko der Resistenzentwicklung zu gewährleisten. IWM-Maßnahmen implizieren jedoch eine erhöhte Systemkomplexität, was ihre Akzeptanz durch die Landwirte erschweren kann. Die Ergebnisse dieser Studie zeigen, dass Cinmethylin in Kombination mit verschiedenen Stoppelbearbeitungsmethoden, Glyphosatanwendungen, Spätsaat oder Herbizid Sequenzen/-mischungen zu nachhaltigen Bekämpfungserfolg führt. Cinmethlyin stellt mit seiner neuartigen und einzigartigen Wirkungsweise eine wertvolle Ergänzung für ein integriertes Unkraut- und Resistenzmanagement dar.

6. REFERENCES

AMANN A, ZWERGER P & HURLE K (1992) Einfluss von Saattermin und Grundbodenbearbeitung auf die Populationsdynamik von Alopecurus myosuroides Huds. in verschiedenen Kulturen. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz,* 269–276.

BAILLY GC, DALE RP, ARCHER SA, WRIGHT DJ & KAUNDUN SS (2012) Role of residual herbicides for the management of multiple herbicide resistance to ACCase and ALS inhibitors in a black-grass population. *Crop Protection* **34,** 96–103.

BÀRBERI P, BARZMAN M, BIRCH ANE et al. (2015) Eight principles of integrated pest management. *Agronomy for Sustainable Development* **35,** 1199–1215.

BARTH JAC, STEIDLE D, KUNTZ D et al. (2007) Deposition, persistence and turnover of pollutants: First results from the EU project AquaTerra for selected river basins and aquifers. *The Science of the total environment* **376,** 40–50.

BASKIN JM & BASKIN CC (1985) The Annual Dormancy Cycle in Buried Weed Seeds: A Continuum. *BioScience* **35,** 492–498.

BECKIE HJ (2006) Herbicide-Resistant Weeds: Management Tactics and Practices. *Weed Technology* **20,** 793–814.

BECKIE HJ, HEAP IM, SMEDA RJ & HALL LM (2000) Screening for Herbicide Resistance in Weeds 1. *Weed Technology* **14,** 428–445.

BERTHOLDSSON N-O (2011) Use of multivariate statistics to separate allelopathic and competitive factors influencing weed suppression ability in winter wheat. *Weed Research* **51,** 273–283.

BLACKSHAW RE, HARKER KN, O'DONOVAN JT, BECKIE HJ & SMITH EG (2008) Ongoing Development of Integrated Weed Management Systems on the Canadian Prairies. *Weed Science* **56,** 146–150.

BLANCO-CANQUI H, SHAVER TM, LINDQUIST JL et al. (2015) Cover Crops and Ecosystem Services: Insights from Studies in Temperate Soils. *Agronomy Journal* **107,** 2449–2474.

BOND W & GRUNDY AC (2001) Non-chemical weed management in organic farming systems. *Weed Research* **41,** 383–405.

BOUTSALIS P (2001) Syngenta Quick-Test. A Rapid Whole-Plant Test for Herbicide Resistance. *Weed Technology* **15,** 257–263.

BRANDSAETER LO, MANGERUD K & RASMUSSEN J (2012) Interactions between pre- and post-emergence weed harrowing in spring cereals. *Weed Research* **52,** 338–347.

BRENNAN EB & SMITH RF (2005) Winter Cover Crop Growth and Weed Suppression on the Central Coast of California. *Weed Technology* **19,** 1017–1024.

BRUST J, CLAUPEIN W & GERHARDS R (2014) Growth and weed suppression ability of common and new cover crops in Germany. *Crop Protection* **63,** 1–8.

BRUST J, GERHARDS R, KARANISA T, RUFF L & KIPP A (2011) Why undersown and cover crops become important again for weed suppression in European cropping systems. *Gesunde Pflanzen* **63,** 191–198.

BUHLER DD (1991) Early Preplant Atrazine and Metolachlor in Conservation Tillage Corn (Zea mays). *Weed Technology* **5,** 66–71.

BUHLER DD (2002) 50th Anniversary—Invited Article: Challenges and opportunities for integrated weed management. *Weed Science* **50,** 273–280.

BURGOS NR, TRANEL PJ, STREIBIG JC et al. (2013) Review: Confirmation of Resistance to Herbicides and Evaluation of Resistance Levels. *Weed Science* **61,** 4–20.

BUSI R, DAYAN FE, FRANCIS I et al. (2020) Cinmethylin controls multiple herbicide-resistant Lolium rigidum and its wheat selectivity is P450-based. *Pest management science* **76,** 2601–2608.

CAMPE R, HOLLENBACH E, KÄMMERER L et al. (2018) A new herbicidal site of action: Cinmethylin binds to acyl-ACP thioesterase and inhibits plant fatty acid biosynthesis. *Pesticide biochemistry and physiology* **148,** 116–125.

CARVALHO FP (2006) Agriculture, pesticides, food security and food safety. *Environmental Science & Policy* **9,** 685–692.

CHAUHAN BS, GILL GS & PRESTON C (2006) Tillage system effects on weed ecology, herbicide activity and persistence: a review. *Australian Journal of Experimental Agriculture* **46,** 1557.

CHAUVEL B, GUILLEMIN J, COLBACH N & GASQUEZ J (2001) Evaluation of cropping systems for management of herbicide-resistant populations of blackgrass (Alopecurus myosuroides Huds.). *Crop Protection* **20,** 127–137.

CHAUVEL B, GUILLEMIN J-P & COLBACH N (2009) Evolution of a herbicide-resistant population of Alopecurus myosuroides Huds. in a long-term cropping system experiment. *Crop Protection* **28.,** 343–349.

COLBACH N, CHAUVEL B, DURR C & RICHARD G (2002a) Effect of environmental conditions on Alopecurus myosuroides germination. I. Effect of temperature and light. *Weed Research* **42,** 210–221.

COLBACH N, DURR C, CHAUVEL B & RICHARD G (2002b) Effect of environmental conditions on Alopecurus myosuroides germination. II. Effect of moisture conditions and storage length. *Weed Research* **42,** 222–230.

COLBACH N, DURR C, ROGER-ESTRADE J & CANEILL J (2005) How to model the effects of farming practices on weed emergence. *Weed Research* **45,** 2–17.

COLBACH N & DÜRR C (2003) Effects of seed production and storage conditions on blackgrass (Alopecurus myosuroides) germination and shoot elongation. *Weed Science* **51,** 708–717.

COLBACH N, DÜRR C, ROGER-ESTRADE J, CHAUVEL B & CANEILL J (2006) AlomySys: Modelling black-grass (Alopecurus myosuroides Huds.) germination and emergence, in interaction with seed characteristics, tillage and soil climate. *European Journal of Agronomy* **24,** 95–112.

CONCENÇO G, SALTON J, SECRETTI M, MENDES P, BREVILIERI R & GALON L (2011) Effect of long-term agricultural management systems on occurrence and composition of weed species. *Planta Daninha* **29,** 515–522.

CONSTANTIN J, LE BAS C & JUSTES E (2015) Large-scale assessment of optimal emergence and destruction dates for cover crops to reduce nitrate leaching in temperate conditions using the STICS soil–crop model. *European Journal of Agronomy* **69,** 75–87.

COOK SK & BROOKE R (2006) Collection and dormancy testing of black-grass seed.

CREAMER NG, BENNETT MA, STINNER BR, CARDINA J & REGNIER EE (1996) Mechanisms of Weed Suppression in Cover Crop-based Production Systems. *HortScience* **31,** 410–413.

CROPMONITOR (2017) Defra Winter Wheat Disease Survey (Source: Fera Science Ltd). http://www.cropmonitor.co.uk/wwheat/surveys/surveygraphs/wheatresult.cfm.

DAMALAS CA & KOUTROUBAS SD (2016) Farmers' Exposure to Pesticides: Toxicity Types and Ways of Prevention. *Toxics* **4**.

DAYAN FE (2019) Current Status and Future Prospects in Herbicide Discovery. *Plants (Basel, Switzerland)* **8**.

DÉLYE C, JASIENIUK M & LE CORRE V (2013) Deciphering the evolution of herbicide resistance in weeds. *Trends in genetics : TIG* **29,** 649–658.

DÉLYE C, MENCHARI Y, GUILLEMIN J-P et al. (2007) Status of black grass (Alopecurus myosuroides) resistance to acetyl-coenzyme A carboxylase inhibitors in France. *Weed Research* **47,** 95–105.

DING G, LIU X, HERBERT S, NOVAK J, AMARASIRIWARDENA D & XING B (2006) Effect of cover crop management on soil organic matter. *Geoderma* **130,** 229–239.

DROBNY HG, SALAS M & CLAUDE J (2006) Management of metabolic resistant black-grass (Alopecurus myosuroides Huds.) populations in Germany-challenges and opportunities. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **20,** 65.

DÜCKER R, ZÖLLNER P, PARCHARIDOU E, RIES S, LORENTZ L & BEFFA R (2019) Enhanced metabolism causes reduced flufenacet sensitivity in black-grass (Alopecurus myosuroides Huds.) field populations. *Pest management science* **75,** 2996–3004.

DUKE SO (2012) Why have no new herbicide modes of action appeared in recent years? *Pest management science* **68,** 505–512.

EPPO (2015) PP 1/213 (4) Resistance risk analysis. *EPPO Bulletin* **45,** 371–387.

ERBACH DC & LOVELY WG (1975) Effect of plant residue on herbicide performance in no-tillage corn. *Weed Science,* 512–515.

FAROOQ M, JABRAN K, CHEEMA ZA, WAHID A & SIDDIQUE KHM (2011) The role of allelopathy in agricultural pest management. *Pest management science* **67,** 493–506.

FISK JW, HESTERMAN OB, SHRESTHA A et al. (2001) Weed Suppression by Annual Legume Cover Crops in No-Tillage Corn. *Agronomy Journal* **93,** 319–325.

GABA S, GABRIEL E, CHADŒUF J, BONNEU F & BRETAGNOLLE V (2016) Herbicides do not ensure for higher wheat yield, but eliminate rare plant species. *Scientific reports* **6,** 30112.

GALLAGHER RS & CARDINA J (1998) The effect of light environment during tillage on the recruitment of various summer annuals. *Weed Science* **46,** 214–216.

GERHARDS R, KOLLENDA B, MACHLEB J et al. (2020) Camera-guided Weed Hoeing in Winter Cereals with Narrow Row Distance. *Gesunde Pflanzen* **72,** 403–411.

GERHARDS R & SCHAPPERT A (2020) Advancing cover cropping in temperate integrated weed management. *Pest management science* **76,** 42–46.

GERHARDS R, SPÄTH M, SÖKEFELD M, PETEINATOS GG, NABOUT A & RUEDA AYALA V (2021) Automatic adjustment of harrowing intensity in cereals using digital image analysis. *Weed Research* **61,** 68–77.

GRUBER S & CLAUPEIN W (2009) Effect of tillage intensity on weed infestation in organic farming. *Soil and Tillage Research* **105,** 104–111.

HARKER KN & O'DONOVAN JT (2013) Recent Weed Control, Weed Management, and Integrated Weed Management. *Weed Technology* **27,** 1–11.

HARTWIG NL & AMMON HU (2002) Cover crops and living mulches. *Weed Science* **50,** 688–699.

HAY JR (1974) Gains to the grower from weed science. *Weed Science* **22,** 439–442.

HEAP I (2014) Herbicide Resistant Weeds. In: *Pesticide problems* (eds. D PIMENTEL & R PESHIN), 281–301. Springer Netherlands, Dordrecht.

HEAP I (2021) The International Herbicide-Resistant Weed Database. www.weedscience.org, last accessed January 27.

HEAP I & KNIGHT R (1982) A population of ryegrass tolerant to the herbicide diclofop-methyl [in South Australia]. *Journal of the Australian Institute of Agricultural Science* **48,** 156–157.

HEAP IM (1997) The occurrence of herbicide-resistant weeds worldwide. *Pesticide Science* **51,** 235–243.

HILLOCKS RJ (2012) Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection* **31,** 85–93.

HOAD S, TOPP C & DAVIES K (2008) Selection of cereals for weed suppression in organic agriculture: a method based on cultivar sensitivity to weed growth. *Euphytica* **163,** 355–366.

HOLT JS (1992) History of Identification of Herbicide-Resistant Weeds. *Weed Technology* **6,** 615–620.

HOLZNER W & NUMATA M, eds (1982) Biology and ecology of weeds. Springer Netherlands, Dordrecht.

HOME MCW, TILLETT ND, HAGUE T & GODWIN RJ (2002) An experimental study of lateral positional accuracy achieved during inter-row cultivation.

HOOKER KV, COXON CE, HACKETT R, KIRWAN LE, O'KEEFFE E & RICHARDS KG (2008) Evaluation of cover crop and reduced cultivation for reducing nitrate leaching in Ireland. *Journal of environmental quality* **37,** 138–145.

JENSEN PK (2009) Longevity of seeds of four annual grass and two dicotyledon weed species as related to placement in the soil and straw disposal technique. *Weed Research* **49,** 592–601.

KAISER YI, MENEGAT A, GERHARDS R & KUDSK P (2013) Chlorophyll fluorescence imaging. A new method for rapid detection of herbicide resistance in Alopecurus myosuroides. *Weed Research* **53,** 399–406.

KAUNDUN SS (2014) Resistance to acetyl-CoA carboxylase-inhibiting herbicides. *Pest management science* **70,** 1405–1417.

KAUNDUN SS, HUTCHINGS S-J, DALE RP, BAILLY GC & GLANFIELD P (2011) Syngenta ‘RISQ’ test. A novel in-season method for detecting resistance to post-emergence ACCase and ALS inhibitor herbicides in grass weeds. *Weed Research* **51,** 284–293.

KNAB W & HURLE K (1988) Effect of primary cultivation on black-grass (Alopecurus myosuroides Huds.). *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*.

KORTEKAMP A, ed (2011) Herbicides and Environment. Intech Open, London, UK.

KRAEHMER H, LABER B, ROSINGER C & SCHULZ A (2014) Herbicides as weed control agents: state of the art: I. Weed control research and safener technology: the path to modern agriculture. *Plant physiology* **166,** 1119–1131.

KUNZ C, STURM DJ, VARNHOLT D, WALKER F & GERHARDS R (2016) Allelopathic effects and weed suppressive ability of cover crops. *Plant, Soil and Environment* **62,** 60–66.

KURSTJENS DAG & KROPFF MJ (2001) The impact of uprooting and soil-covering on the effectiveness of weed harrowing. *Weed Research* **41,** 211–228.

LÖTJÖNEN T & MIKKOLA HJ (2000) Three mechanical weed control techniques in spring cereals. *Agricultural and Food Science* **9,** 269–278.

LUTMAN PJW, FREEMAN SE & 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.

LUTMAN PJW, MOSS SR, COOK S & WELHAM SJ (2013) A review of the effects of crop agronomy on the management of Alopecurus myosuroides. *Weed Research* **53,** 299–313.

MACHLEB J, KOLLENDA BL, PETEINATOS GG & GERHARDS R (2018) Adjustment of Weed Hoeing to Narrowly Spaced Cereals. *Agriculture* **8,** 54.

MACHLEB J, PETEINATOS GG, KOLLENDA BL, ANDÚJAR D & GERHARDS R (2020) Sensor-based mechanical weed control: Present state and prospects. *Computers and Electronics in Agriculture* **176,** 105638.

MELANDER & RASMUSSEN (2000) Reducing intrarow weed numbers in row crops by means of a biennial cultivation system. *Weed Research* **40,** 205–218.

MELANDER B (1995) Impact of drilling date on Apera spica-venti L. and Alopecurus myosuroides Huds, in winter cereals. *Weed Research* **35,** 157–166.

MELANDER B, HOLST N, JENSEN PK, HANSEN EM & OLESEN JE (2008) Apera spica-venti population dynamics and impact on crop yield as affected by tillage, crop rotation, location and herbicide programmes. *Weed Research* **48,** 48–57.

MELANDER B, HOLST N, RASMUSSEN IA & HANSEN PK (2012) Direct control of perennial weeds between crops – Implications for organic farming. *Crop Protection* **40,** 36–42.

MELANDER B, MUNIER-JOLAIN N, CHARLES R et al. (2013) European Perspectives on the Adoption of Nonchemical Weed Management in Reduced-Tillage Systems for Arable Crops. *Weed Technology* **27,** 231–240.

MELANDER B, RASMUSSEN IA & BÀRBERI P (2005) Integrating physical and cultural methods of weed control— examples from European research. *Weed Science* **53,** 369–381.

MENCHARI Y, CAMILLERI C, MICHEL S et al. (2006) Weed response to herbicides: regional-scale distribution of herbicide resistance alleles in the grass weed Alopecurus myosuroides. *The New phytologist* **171,** 861–873.

MENCHARI Y, DÉLYE C & LE CORRE V (2007) Genetic variation and population structure in black-grass (Alopecurus myosuroides Huds.), a successful, herbicide-resistant, annual grass weed of winter cereal fields. *Molecular ecology* **16,** 3161–3172.

MENEGAT A & NILSSON ATS (2019) Interaction of Preventive, Cultural, and Direct Methods for Integrated Weed Management in Winter Wheat. *Agronomy* **9,** 564.

MENNE HJ & HOGREFE C (2012) Impact of multiple resistance mechanisms in black-grass (Alopecurus myosuroides Huds.) populations on the of cereal herbicides. Julius-Kühn-Archiv; 434 (1); 65-74 / Julius-Kühn-Archiv; 434 (1); 65-74. *Julius-Kühn-Archiv* **1,** 65–74.

MENNE HJ, LABER B, KERLEN D & BEFFA R, eds (2012) Effectiveness of flufenacet in controlling resistant black-grass (Alopecurus myosuroides Huds.) - comparison of glasshouse and field trial results.

MERFIELD CN (2015) False and Stale Seedbeds: The most effective non-chemical weed management tools for cropping and pasture establishment, 25th edn. The BHU Future Farming Centre, Lincoln, New Zealand.

MIRSKY SB, GALLANDT ER, MORTENSEN DA, CURRAN WS & SHUMWAY DL (2010) Reducing the germinable weed seedbank with soil disturbance and cover crops. *Weed Research* **50,** no-no.

MOONEN AC & BARBERI P (2004) Size and composition of the weed seedbank after 7 years of different cover-crop-maize management systems. *Weed Research* **44,** 163–177.

MOSS S (2017) Black-grass (Alopecurus myosuroides): Why has this Weed become such a Problem in Western Europe and what are the Solutions? *Outlooks on Pest Management* **28,** 207–212.

MOSS SR (1980) The agro-ecology and control of black-grass, Alopecurus myosuroides Huds., in modern cereal growing systems. *ADAS Quarterly Review,* 170–191.

MOSS SR (1985) The survival of Alopecurus myosuroides Huds. seeds in soil. *Weed Research* **25,** 201–211.

MOSS SR, ed (1990) The seed cycle of Alopecurus myosuroides in winter cereals: a quantitative analysis. European Weed Research Society, Helsinki, Finland.

MOSS SR (1995) Techniques for determining herbicide resistance. *Brighton Corp Prot Conf Weeds,* 547–556.

MOSS SR (2010a) Integrated weed management (IWM): will it reduce herbicide use? *Communications in agricultural and applied biological sciences* **75,** 9–17.

MOSS SR, ed (2010b) Non-chemical methods of weed control: benefits and limitations.

MOSS SR & CLARKE JH (1994) Guidelines for the prevention and control of herbicide-resistant black-grass (Alopecurus myosuroides Huds.). *Crop Protection* **13,** 230–234.

MOSS SR & HULL R (2009) The value of pre-emergence herbicides for combating herbicide-resistant Alopecurus myosuroides (black-grass). *Aspects of Applied Biology* **83,** 109–113.

MOSS SR, PERRYMAN SA & TATNELL LV (2007) Managing Herbicide-resistant Blackgrass (Alopecurus Myosuroides): Theory and Practice. *Weed Technology* **21,** 300–309.

NATIONAL RESEARCH COUNCIL, ed (1929) Proceedings of a conference on the destruction of weeds by means of chemicals.

NAYLOR RE (1970) The prediction of black-grass infestations. *Weed Research* **10,** 296–299.

NEVE P (2007) Challenges for herbicide resistance evolution and management: 50 years after Harper. *Weed Research* **47,** 365–369.

NICHOLS V, VERHULST N, COX R & GOVAERTS B (2015) Weed dynamics and conservation agriculture principles: A review. *Field Crops Research* **183,** 56–68.

OECD (2006) Guideline for the testing of chemicals, section 2 test no. 208: Terrestrial plant test:seedling emergence and seedling growth test.

OERKE E-C (2006) Crop losses to pests. *The Journal of Agricultural Science* **144,** 31–43.

OLIPHANT J.M. (1977) Some notes on effect of herbicide use and cultivation practice on population and control of Avena spp. in winter wheat. *Experimental Husbandry*.

ORSON JH (1996) The sustainability of intensive arable systems: implications for rotational policy. *Aspects of Applied Biology*.

PAGLIAI M, VIGNOZZI N & PELLEGRINI S (2004) Soil structure and the effect of management practices. *Soil and Tillage Research* **79,** 131–143.

PEKRUN C & CLAUPEIN W (2006) The implication of stubble tillage for weed population dynamics in organic farming. *Weed Research* **46,** 414–423.

PEKRUN C, HEWITT JDJ & LUTMAN PJW (1998) Cultural control of volunteer oilseed rape (Brassica napus). *The Journal of Agricultural Science* **130,** 155–163.

POWLES SB & SHANER DL, eds (2001) Herbicide resistance and world grains. CRC Press, Boca Raton, Fla.

POWLES SB & YU Q (2010) Evolution in action: plants resistant to herbicides. *Annual review of plant biology* **61,** 317–347.

PRADO R de, JORRÍN J & GARCÍA-TORRES L, eds (1997) Weed and crop resistance to herbicides. Springer, Dordrecht, Boston.

PRUEGER JH, ALFIERI J, GISH TJ et al. (2017) Multi-Year Measurements of Field-Scale Metolachlor Volatilization. *Water, Air, & Soil Pollution* **228**.

RASMUSSEN IA (2004) The effect of sowing date, stale seedbed, row width and mechanical weed control on weeds and yields of organic winter wheat. *Weed Research* **44,** 12–20.

RASMUSSEN J (1992) Testing harrows for mechanical control of annual weeds in agricultural crops. *Weed Research* **32,** 267–274.

RASMUSSEN J, BIBBY BM & SCHOU AP (2008) Investigating the selectivity of weed harrowing with new methods. *Weed Research* **48,** 523–532.

RASMUSSEN J, NIELSEN HH & GUNDERSEN H (2009) Tolerance and Selectivity of Cereal Species and Cultivars to Postemergence Weed Harrowing. *Weed Science* **57,** 338–345.

RASMUSSEN J & SVENNINGSEN T (1995) Selective Weed Harrowing in Cereals. *Biological Agriculture & Horticulture* **12,** 29–46.

RAY TB (1984) Site of action of chlorsulfuron: inhibition of valine and isoleucine biosynthesis in plants. *Plant physiology* **75,** 827–831.

READE JPH & COBB AH (2002) New, quick tests for herbicide resistance in black-grass (Alopecurus myosuroides Huds) based on increased glutathione S-transferase activity and abundance. *Pest management science* **58,** 26–32.

ROSENHAUER M & PETERSEN J (2015) Bioassay Development for the Identification of Pre-emergence Herbicide Resistance in Alopecurus myosuroides (Huds.) Populations. *Gesunde Pflanzen* **67,** 141–150.

RYAN GF (1970) Resistance of Common Groundsel to Simazine and Atrazine. *Weed Science* **18,** 614–616.

RYDBERG T (1994) Weed Harrowing—the Influence of Driving Speed and Driving Direction on Degree of Soil Covering and the Growth of Weed and Crop Plants. *Biological Agriculture & Horticulture* **10,** 197–205.

SAARI LL (1994) Resistance to acetolactate synthase inhibiting herbicides. *Herbicide Resistance in Plants : Biology and Biochemistry,* 83–139.

SARDANA V, MAHAJAN G, JABRAN K & CHAUHAN BS (2017) Role of competition in managing weeds: An introduction to the special issue. *Crop Protection* **95,** 1–7.

SCHAPPERT A, LINN AI, STURM DJ & GERHARDS R (2019) Weed suppressive ability of cover crops under water-limited conditions. *Plant, Soil and Environment* **65,** 541–548.

SCHAPPERT A, MESSELHÄUSER M, SAILE M, PETEINATOS G & GERHARDS R (2018) Weed Suppressive Ability of Cover Crop Mixtures Compared to Repeated Stubble Tillage and Glyphosate Treatments. *Agriculture* **8,** 144.

SCHERNER A, MELANDER B, JENSEN PK, KUDSK P & AVILA LA (2017) Germination of Winter Annual Grass Weeds under a Range of Temperatures and Water Potentials. *Weed Science* **65,** 468–478.

SEAVERS GP & WRIGHT KJ (1999) Crop canopy development and structure influence weed suppression. *Weed Research* **39,** 319–328.

SHIFERAW B, SMALE M, BRAUN H-J, DUVEILLER E, REYNOLDS M & MURICHO G (2013) Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Security* **5,** 291–317.

SPAETH M, MACHLEB J, PETEINATOS GG, SAILE M & GERHARDS R (2020) Smart Harrowing—Adjusting the Treatment Intensity Based on Machine Vision to Achieve a Uniform Weed Control Selectivity under Heterogeneous Field Conditions. *Agronomy* **10,** 1925.

STEWART CL, SOLTANI N, NURSE RE, HAMILL AS & SIKKEMA PH (2012) Precipitation Influences Pre- and Post-Emergence Herbicide Efficacy in Corn. *American Journal of Plant Sciences* **03,** 1193–1204.

SWAIN AJ, HUGHES ZS, COOK SK & MOSS SR (2006) Quantifying the dormancy of Alopecurus myosuroides seeds produced by plants exposed to different soil moisture and temperature regimes. *Weed Research* **46,** 470–479.

SWANTON CJ & MURPHY SD (1996) Weed Science Beyond the Weeds: The Role of Integrated Weed Management (IWM) in Agroecosystem Health. *Weed Science* **44,** 437–445.

SWANTON CJ & WEISE SF (1991) Integrated Weed Management: The Rationale and Approach. *Weed Technology* **5,** 657–663.

TEASDALE JR (1996) Contribution of Cover Crops to Weed Management in Sustainable Agricultural Systems. *Journal of Production Agriculture* **9,** 475–479.

(2017) The future of food and agriculture. Trends and challenges. Food and Agriculture Organization of the United Nations, Rome.

TILLMAN G, SCHOMBERG H, PHATAK S et al. (2004) Influence of Cover Crops on Insect Pests and Predators in Conservation Tillage Cotton. *Journal of Economic Entomology* **97,** 1217–1232.

TRAVLOS I, GAZOULIS I, KANATAS P, TSEKOURA A, ZANNOPOULOS S & PAPASTYLIANOU P (2020) Key Factors Affecting Weed Seeds' Germination, Weed Emergence, and Their Possible Role for the Efficacy of False Seedbed Technique as Weed Management Practice. *Frontiers in Agronomy* **2**.

TROYER JR (2001) In the beginning: the multiple discovery of the first hormone herbicides. *Weed Science* **49,** 290–297.

VAN DER WEIDE RY, BLEEKER PO, ACHTEN VTJM, LOTZ LAP, FOGELBERG F & MELANDER B (2008) Innovation in mechanical weed control in crop rows. *Weed Research* **48,** 215–224.

VARANASI A, PRASAD PV & JUGULAM M (2016) Impact of Climate Change Factors on Weeds and Herbicide Efficacy. In: *Advances in Agronomy* (ed. DL SPARKS), 107–146. Elsevier Science, s.l.

WILTSHIRE JJJ, TILLETT ND & HAGUE T (2003) Agronomic evaluation of precise mechanical hoeing and chemical weed control in sugar beet. *Weed Research* **43,** 236–244.

WISLER GC & NORRIS RF (2005) Interactions between weeds and cultivated plants as related to management of plant pathogens. *Weed Science* **53,** 914–917.

WRUCKE MA & ARNOLD WE (1985) Weed Species Distribution as Influenced by Tillage and Herbicides. *Weed Science* **33,** 853–856.

WYSE DL (1992) Future of Weed Science Research. *Weed Technology* **6,** 162–165.

ZELLER A, KAISER Y & GERHARDS R (2018) Suppressing Alopecurus myosuroides Huds. in Rotations of Winter-Annual and Spring Crops. *Agriculture* **8,** 91.

ZELLER AK, ZELLER YI & GERHARDS R (2021) A long-term study of crop rotations, herbicide strategies and tillage practices: Effects on Alopecurus myosuroides Huds. Abundance and contribution margins of the cropping systems. *Crop Protection* **145,** 105613.

ZERNER MC, GILL GS & VANDELEUR RK (2008) Effect of Height on the Competitive Ability of Wheat with Oats. *Agronomy Journal* **100,** 1729–1734.

ZIMDAHL RL (2018) Fundamentals of weed science. https://www.sciencedirect.com/science/book/9780128111437.

ZIMDAHL RL & CLARK SK (1982) Degradation of Three Acetanilide Herbicides in Soil. *Weed Science* **30,** 545–548.

ZIMMERMANN C, PEKRUN C, SCHIEFER R et al. (2011) Zwischenfrüchte mit dem Mähdrescher säen. Erste Erfahrunge mit der Mähdruschsaat. *Innovation* **2**.

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8. DECLARATION IN LIEU OF AN OATH ON INDEPENDENT WORK

according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic

"Incorporating agronomic measures into integrated weed management strategies using pre-emergence herbicide cinmethylin to control *Alopecurus myosuroides* Huds."

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

U. Messelhäuser

Hohenheim, 24.12.21

Place, Date Signature

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