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Suppressing *Alopecurus myosuroides* Huds. in Rotations of Winter-Annual and Spring Crops

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Abstract: *Alopecurus myosuroides* Huds. has become one of the most abundant grass weeds in Europe. High percentages of winter-annual crops in the rotation, earlier sowing of winter wheat and non-inversion tillage favor *A. myosuroides*. Additionally, many populations in Europe have developed resistance to acetyl-CoA carboxylase (ACCase), acetolactate synthase (ALS) and photosynthetic (PSII) inhibitors. Hence, yield losses due to *A. myosuroides* have increased. On-farm studies have been carried out in Southern Germany over five years to investigate abundance, control efficacies and crop yield losses due to *A. myosuroides*. Three crop rotations were established with varying proportions of winter- and summer-annual crops. The crop rotations had a share of 0, 25 and 50% of summer-annual crops. Within each crop rotation, three herbicide strategies were tested. In contrast to classical herbicidal mixtures and sequences, the aim of one of the herbicide strategies was to keep selection pressure as low as possible by using each mode of action (MOA) only once during the five years. *A. myosuroides* population was susceptible to all herbicide at the beginning of the experiment. Initial average density was 14 plants m⁻². In the rotation with only winter-annual crops, density increased to 5347 ears m⁻² in the untreated control plots. Densities were lower in the rotations with 25% and even lower with 50% summer-annual crops. Control efficacies against *A. myosuroides* in the herbicide strategy using only MOAs of the HRAC-groups B and A, according to the Herbicide Resistance Action Committee (HRAC) classification on MOA, dropped after five years compared to the strategy of changing MOA in every year. Nevertheless, the results demonstrate the need for combining preventive and direct weed-management strategies to suppress *A. myosuroides* and maintain high weed-control efficacies of the herbicides.

Keywords: mode of action (MOA); preventive weed control; herbicide resistance management; crop rotation; herbicide rotation

1. Introduction

Crop rotations can be very effective at controlling weeds in Integrated Weed Management (IWM) [1]. However, crop diversity has decreased by 50–70% in European cropping systems within the past 50 years. This is due to the use of synthetic fertilizers and pesticides [2]. Production of spring cereals, potatoes, fiber crops and legumes has decreased, whereas production of winter cereals, oilseed rape and maize has increased. Winter wheat, winter barley and winter oilseed rape are dominant in moderate and humid areas with often 75–100% winter-annual crops in the rotations [3]. Winter cereals realizes higher yield output than spring cereals and achieve higher contribution margins [4]. The combination of cost reduction due to a minimized cultivation and a herbicide-related system used as described by Power and Follet [5], made the system sustainable.

Alopecurus myosuroides Huds. is a winter-annual weed predominantly germinating in autumn [6]. It prefers heavy, loamy, and waterlogged soils. In Western Europe, *A. myosuroides* has become very

abundant in winter wheat and winter oilseed rape, particularly in early sown winter cereals after reduced tillage practices [7–9]. *A. myosuroides* produces about 100 seeds per ear with a lifetime of up to 10 years [8]. It is a very competitive grass weed in winter wheat with 100 plants m^{-2} resulting in crop yield losses of approximately 20% [10–12]. Infestation rates of 500 plants m^{-2} cause yield losses of up to 50% [9–12].

Due to continuous applications of herbicides with the same modes of action (MOA), there has been a selection for herbicide-resistant weed populations [13]. Because of widespread evolved resistances, a lot of *A. myosuroides* populations have survived standard herbicide applications. Therefore, *A. myosuroides* has become the most problematic weed species in Europe [10]. Populations with evolved resistance to herbicides have been documented in almost all European countries. Resistances to ACCase-, ALS- and PS2-inhibitors are widespread in Germany [14]. Several *A. myosuroides* populations showed cross- and multiple-resistances [15]. Nevertheless, farmers prefer cultivating winter wheat because it provides higher contribution margins than spring cereals [16]. They usually start resistance management once the problem has become very evident.

There are studies that highlight the influence of spring barley on *A. myosuroides* densities [9,11,17]. Furthermore, recent studies have investigated the effect of herbicide mixtures and sequences, which are intended to prevent resistance development by using different MOA [18]. In our five-year study, we demonstrate the long-term effect on *A. myosuroides* densities by summer-annual crops, and the influence of different proportions of summer-annual crops in the crop rotation. Furthermore, we show the combination and interaction between different herbicide strategies. Additionally, we deviate from typical herbicide mixtures and sequences by setting the focus on minimal selection pressure, and not on the herbicide efficacy of *A. myosuroides* as usual.

We tested, (1) how much summer-annual crops in a rotation can reduce *A. myosuroides* densities compared to typical winter-annual cropping systems under conditions in Southern Germany. For this purpose, three different crop rotations were carried out, which differed in their proportion of summer-annual crops (0, 25 and 50%). The second hypothesis was (2), that using every herbicide MOA only once over the five-year period would result in higher *A. myosuroides* control efficacies than using herbicides of the HRAC-groups B and A every year, in the long term. Finally (3), we assumed that only through the combination of preventive measures—crop rotation—and minimal selection pressure—annual MOA change—could a satisfactory result be achieved.

2. Materials and Methods

2.1. Field Experiment and Trial Design

The experiment was designed as a randomized split-plot. The main plot factor was crop rotation (CR) and the sub-plot factor herbicide strategy (HS). The size of each sub-plot was 6 m \times 12 m. Each combination of CR \times HS was replicated 4 times. In CR1, 3 years of winter wheat (*Triticum aestivum* L.) was followed by winter oilseed rape (*Brassica napus* L.). In CR2, winter wheat in the 3rd year was replaced by spring barley (*Hordeum vulgare* L.) and in CR3, winter wheat in the 2nd year was replaced by maize (*Zea mays* L.), and spring barley was grown in the 3rd year. In Year 5, crop rotation started again with winter wheat in all variances. The experimental design was set up to test different proportions of summer-annual crops in the rotations (CR1 0%, CR2 25% and CR3 50%) on the infestation of *A. myosuroides*. However, with this experimental design, the results of the experiment are correlated with the annual conditions. Therefore, it was not possible to capture the difference between crop and year separately. Three weed-control strategies were included in each crop and year. HS1 was the untreated control. Only broad-leaved weeds were controlled. In HS2, herbicide MOA was changed in every year to reduce the selection pressure on a single MOA. Therefore, each MOA was used only once over the 5-year experiment. To ensure this, active ingredients were also used which are known to have only weak efficacies to *A. myosuroides* or high dependence on weather conditions. In HS3, the selection pressure was high, and we tried to use only herbicides of HRAC-group B. If this

was not possible due to the cultivated crop (spring barley and winter oilseed rape), herbicides of HRAC-group A were used, which are also among the highly effective but also high resistance-risk MOA (Table 1). Broad-leaved weeds were controlled in all herbicide strategies with active ingredients that had no activity against grasses.

Table 1. Crop rotations (CR), sowing dates and herbicide strategies (HS) of the experiment.

Crop Rotation Sowing Date	Year				
	1	2	3	4	5
1	winter wheat 25/10/2011	winter wheat 31/10/2012	winter wheat 19/11/2013	winter oilseed rape 23/08/2014	winter wheat 12/10/2015
2	winter wheat 25/10/2011	winter wheat 31/10/2012	summer barley 19/03/2014	winter oilseed rape 23/08/2014	winter wheat 12/10/2015
3	winter wheat 25/10/2011	maize 25/04/2013	summer barley 19/03/2014	winter oilseed rape 23/08/2014	winter wheat 12/10/2015
Herbicide strategy (HS)					
1	untreated control *				
2	change of herbicide MOA every year				
3	herbicides of the HRAC-groups B and A				

* only broad-leaved weeds were controlled.

The field experiment took place in South-west Germany (48.74° N, 8.92° E, 478 m altitude). The study started in autumn 2011 and continued until summer 2016. In 2011, pea was cultivated before winter wheat. After 3 passes of chisel plough, winter wheat was sown. The average annual temperature is 9.1 °C and the average annual precipitation is 825 mm. The soil texture is a clayey loam. The cultivars Schamane (Saatzucht Streng, Uffenheim, Germany; winter wheat), Torres (KWS, Saat AG, Einbeck, Germany; maize), Grace (Baywa, München, Germany; spring barley) and Avatar (Rapool, Isernhagen HB, Germany; oilseed rape) were sown in the experiment. Sowing dates were adapted to the weather conditions (Table 1). For cereals and oilseed rape, a single disc drill (row distance 0.12 m) and for corn a precision air seeder (row distance 0.75 m) were used. Prior to the spring-crops, lacy phacelia (*Phacelia tanacetifolia* Benth.) was cultivated as a cover crop (cv. Julia, Feldsaaten Freudenberger, Magdeburg, Germany). Glyphosate (1440 g a.i. ha⁻¹, Clinic®, 360 g a.i. L⁻¹, Nufarm Deutschland GmbH, Cologne, Germany) was sprayed to remove over-wintering cover crops, volunteer cereals and weeds. Subsequently, shallow soil tillage was carried out and the spring crops were sown. All crops were fertilized as usual. Fungicides and insecticides were applied if necessary. Throughout the five-year study, only non-inversion tillage operations (typical for south-west Germany) not deeper than 0.12 m were performed. Herbicides were applied with a self-propelled plot sprayer (Schachtner-Gerätetechnik, Ludwigsburg, Germany), which was calibrated for a volume of 200 L ha⁻¹. Crop yield was recorded in 4 × 6 m sub-plots using a plot combine harvester. All herbicides were applied at the recommended dose (Table 2).

Table 2. Herbicides applied in different crops and years to control *A. myosuroides*.

Year	Crop	HS	Application Time	Herbicide (Trade Name)	Active Ingredient	HRAC-Code	Rate (g a.i. ha ⁻¹)
1	WW	2 & 3	spring	Broadway® + Adj. ¹	pyroxsulam + florasulam *	B	15 + 5 *
2	WW	2	spring	Arelon® TOP ³	isoproturon	C1	1500
		3	spring	Broadway® + Adj. ¹	pyroxsulam + florasulam *	B	15 + 5 *
	C	2	spring	Laudis® ²	tembotrione	F2	88
		3	spring	Elumis® ⁵	mesotrione * + nicosulfuron	F2 * + B	93.8 * + 37.5
3	WW	2	autumn; spring	Herold® SC ⁶ ; Traxos® ⁵	flufenacet + diflufenican; clodinafop + pinoxaden	K3 + F1; A	240 + 120; 30 + 30
		3	spring	Broadway® + Adj. ¹	pyroxsulam + florasulam *	B	15 + 5 *
	SB	2	spring	Axial 50® ⁵	pinoxaden	A	60
		3	spring	Axial 50® ⁵	pinoxaden	A	60
4	OR	2	autumn; winter	Butisan® Gold + Stomp® Aqua ⁴ ; Kerb Flo® ¹	dimethenamid * + metazachlor + pendimethalin; propyzamid	K3 * + K3 + K1; K1	400 * + 400 + 341,25; 750
		3	autumn	Gallant® Super ¹	haloxyfop	A	52
5	WW	2	autumn	Boxer® ⁵	prosulfocarb	N	4000
		3	spring	Broadway® + Adj. ¹	pyroxsulam + florasulam *	B	15 + 5 *

Adj. = adjuvant; WW = winter wheat; C = corn; SB = summer barley; OR = winter oilseed rape; * = No efficacy against *A. myosuroides*. ¹ DOW AgroSciences GmbH, Munich, Germany; ² Bayer AG CropScience, Monheim am Rhein, Germany; ³ FMC Corporation Cheminova Deutschland GmbH, Stade, Germany; ⁴ BASF Plant Protection, Ludwigshafen, Germany; ⁵ Syngenta Agro GmbH, Maintal, Germany.

2.2. Assessments of *A. myosuroides* Density and Control Efficacy

The effects of CR and HS on *A. myosuroides* densities were assessed by counting ears of *A. myosuroides* in 5 randomly placed frames (0.4 m²) per plot in each crop and year at flowering stage of the crop. *A. myosuroides* control efficacy (ACE) was calculated using Equation (1):

$$ACE (\%) = \left[\frac{(A - B)}{A} \right] \times 100 \quad (1)$$

where A represents the number of *A. myosuroides* ears m⁻² in the untreated control plots and B represents the number of *A. myosuroides* ears m⁻² in the treated HS plots 14 days after treatment.

2.3. Statistical Analysis

Data analysis was performed with the statistical software R® 3.3.3 (R Development Core Team, Vienna, Austria, 2011). A linear mixed-effect model was used to evaluate the response of *A. myosuroides* to CR and HS as fixed factors. A covariance matrix θ was applied during the analysis of data. Results were log transformed to homogenize variances and to normalize the distribution. In the results section, back transformed means are shown. Crop yield (t ha⁻¹) and ACE (%) were evaluated in each crop and year separately. Yield was analyzed by ANOVA and ACE by linear mixed model. Multiple mean comparison tests were performed using the Tukey test at a significance level of $\alpha \leq 0.05$. Figures were created with Sigmaplot V12.5 (Systat software GmbH, Erkrath, Germany).

3. Results

Crop rotation (CR), herbicide strategy (HS) and year, as well as their interaction, significantly influenced *A. myosuroides* density. *A. myosuroides* density was relatively low at the beginning of the experiment with an average density of 14 ears m⁻². Plants were distributed homogenously within the experiment at the beginning. Infestation rates were below or close to the economic weed thresholds of 10–15 plants m⁻² [19]. During the study, densities significantly increased in all three rotations in the untreated control plots (HS1) (Figures 1 and 2, and Table 3). In the third year of winter wheat in CR1,

A. myosuroides density increased to 883 ears m^{-2} . After five years, a maximum density of 5347 ears m^{-2} was counted in CR1 (Figure 1). When winter wheat was replaced by spring barley in the third year (CR2), densities of *A. myosuroides* were 33% lower than in CR1. In CR3, with maize and spring barley, *A. myosuroides* densities were 50% lower than in CR1, after five years (Figure 1, Table 3).

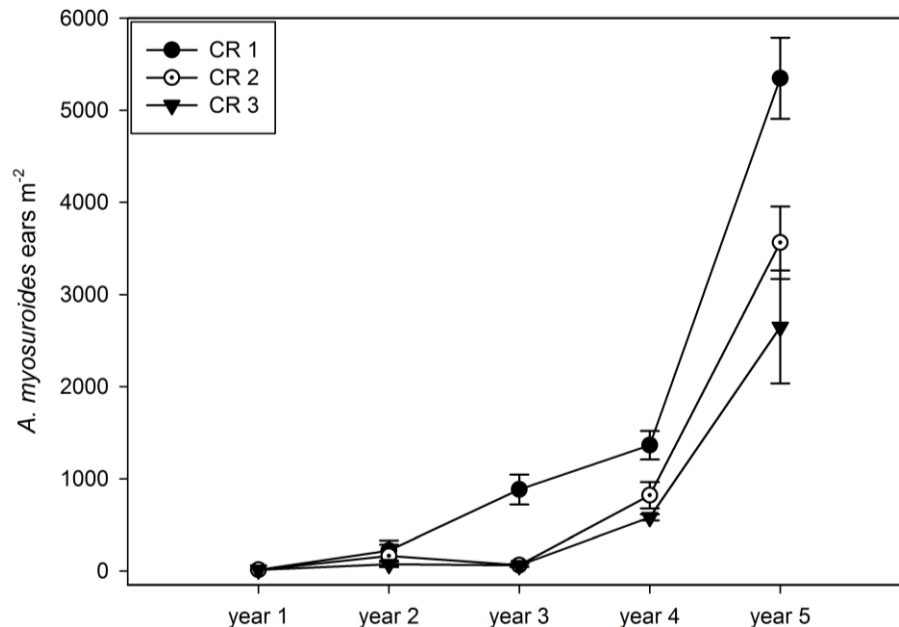


Figure 1. *A. myosuroides* ears m^{-2} in the untreated control plots of crop rotations (CR) 1, 2 and 3 over the five-year experiment at Ihinger Hof, Renningen, Germany.

Herbicide strategies also had a strong impact on *A. myosuroides* densities (Figure 2). In the first three years of the experiment, densities and control efficacies were similar in the strategies using MOA of HRAC-groups B and A and different MOA every year. Weed-control efficacies, weed densities and crop yields were only significantly different from the untreated control. In the second year, ACE ranged between 85%–95% in winter wheat. In maize, HS3 showed the best ACE with 100%, whereas HS2 performed significantly worse with 77%. The reason for the low control efficacy in HS2 was due to the use of tembotrione, which is less active against *A. myosuroides* than nicosulfuron in HS3. Nevertheless, it was applied in this experiment in HS2 to ensure a continuous change of herbicide MOA. *A. myosuroides* infestations in the untreated control caused tremendous yield losses of 81% compared to both herbicide strategies (Table 3).

In contrast to maize, spring barley yield was not reduced by *A. myosuroides* competition. In the third year, spring barley yields were equal in all herbicide strategies and not different to the untreated control, although 65 *A. myosuroides* ears m^{-2} were counted in the control plots. *A. myosuroides* infestation in the third year of winter wheat in CR1 reduced grain yield by 39% compared to HS2 and HS3 (Table 3, Figure 2). Over the period of five years, herbicide efficacy was highest in HS2 (continuous change of herbicide MOA), followed by HS3. From the fourth year onwards, ACE of HS3, using exclusively HRAC-groups B and A herbicides, was significantly lower than in HS2. This resulted in a rapid increase of *A. myosuroides* densities in HS3 in the fifth year of study. Surprisingly, application of only the pre-emergent herbicide prosulfocarb in HS2 resulted in 99% control efficacy against *A. myosuroides*. No post-emergent herbicide was needed in spring. This supports the observations of Naylor [6] that seeds of *A. myosuroides* mostly germinate in autumn. However, efficacy of pre-emergent herbicides could be much lower when the soil is dry after seeding winter-annual crops [7].

Table 3. Average densities of *A. myosuroides* ears m⁻², *A. myosuroides* control efficacy (ACE) and crop yield in the crop rotations (CR) and herbicide strategies (HS).

Year	CR	HS	<i>A. myosuroides</i> Ears m ⁻²	Standard Errors	ACE (%)	Crop Yield (t ha ⁻¹)
1	1; 2; 3—WW	1; 2; 3	14	1.6	99	6.93
2	1—WW	1	222 a	94	—	7.3 a
		2	20 b	14	91 a	8.6 a
		3	12 b	7	95 a	8.4 a
	2—WW	1	166 a	105	—	7.9 a
		2	25 b	14	85 a	8.8 a
		3	16 b	7	90 a	8.2 a
	3—M	1	75 a	20	—	1.5 b
		2	18 b	3	77 b	6.9 a
		3	0 c	0	100 a	7.0 a
3	1—WW	1	883 a	140	—	5.6 b
		2	33 b	25	96 a	9.0 a
		3	6 c	9	99 a	9.3 a
	2—SB	1	65 a	14	—	6.7 a
		2	0 b	0	100 a	6.8 a
		3	1 b	1	99 a	7.0 a
	3—SB	1	63 a	15	—	6.5 a
		2	1 b	0.6	99 a	7.4 a
		3	0 b	0	100 a	6.0 a
4	1—OR	1	1365 a	133	—	0.7 b
		2	1 c	0.6	100 a	5.0 a
		3	81 b	25	94 b	4.4 a
	2—OR	1	822 a	123	—	1.4 b
		2	0 c	0	100 a	4.7 a
		3	56 b	71	93 b	4.6 a
	3—OR	1	582 a	28	—	1.1 b
		2	0 c	0	100 a	4.0 a
		3	44 b	39	93 b	3.7 a
5	1—WW	1	5347 a	381	—	0.8 b
		2	120 c	6	98 a	5.8 a
		3	917 b	466	83 b	4.6 ab
	2—WW	1	3562 a	340	—	0.9 b
		2	77 c	17	98 a	6.0 a
		3	770 b	307	78 b	4.3 ab
	3—WW	1	2648 a	230	—	0.9 b
		2	39 c	6	99 a	5.7 a
		3	366 b	124	86 b	4.1 ab

WW = winter wheat; M =maize, SB = spring barley, OR = winter oilseed rape; Means followed by the same letter are not significantly different according to Tukey's HSD method at the $P = 0.05$ significance level.

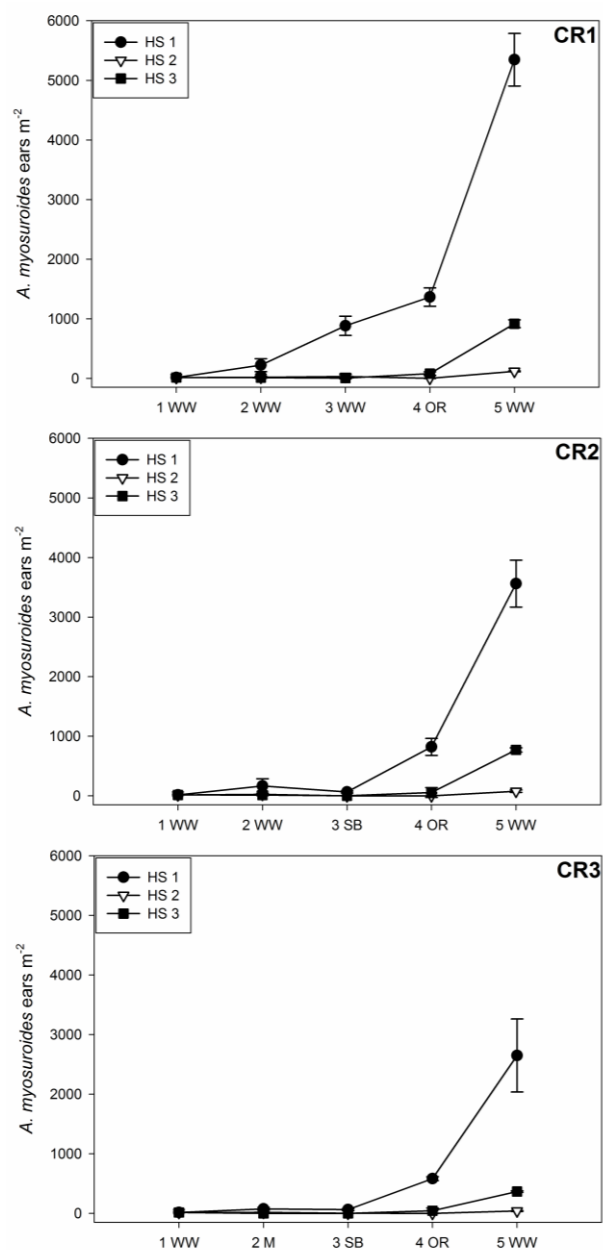


Figure 2. *A. myosuroides* densities expressed in ears m^{-2} at the different herbicide strategies (HS) in crop rotations (CR).

4. Discussion

Alopecurus myosuroides is a very competitive weed species that is well adapted to winter wheat production in Europe. It can rapidly increase population density when no efficient control methods are applied and rotations with a high percentage of winter-annual crops and reduced tillage are practiced. This was shown in this study and it agrees with the results of References [7,8,10,12,17]. Yields of oilseed rape (Year 4) and winter wheat (Year 5) in the untreated plots were approximately 85% lower than in the most effective herbicide treatments (Table 3). These results are in line with Blair et al. [11], who also observed 80% grain yield losses due to *A. myosuroides* competition in winter wheat.

Lutman et al. [9] reported that spring barley reduced *A. myosuroides* densities on average by 88%, because the majority of *A. myosuroides* seeds germinate in autumn [6,20]. In our study, the inclusion of summer-annual crops reduced *A. myosuroides* densities by 61% in Year 2 (CR3) and 93% (CR2 + 3)

in Year 3. After five years, a proportion of 25% summer-annual crops in the rotation (CR2) reduced *A. myosuroides* densities by 33%. With a proportion of 50%, when spring barley and maize were included (CR3) a reduction of 50% could be achieved. Freckleton et al. [17] were also able to demonstrate the positive influence of spring barley on the reduction of *A. myosuroides* densities. They assume that in addition to the main germination in autumn, the tillage and seedbed preparation in the spring remove most of the plants. Secondly, they cite the competitiveness of spring barley, which is characterized by rapid growth and high biomass production, which can suppress the development of *A. myosuroides* effectively. This coincides with our results. We were able to achieve a significant *A. myosuroides* reduction with corn, but only with spring barley yield could be maintained in HS1. Late seeding of winter wheat, rotational ploughing, false seedbed preparation, increased crop density and growing competitive winter wheat cultivars are also efficient preventive methods to suppress *A. myosuroides* [9].

Before the start of the experiment, a greenhouse bioassay with 10 different herbicides of the active ingredient classes HRAC-groups A, B and C was performed. For all herbicides, efficacies between 95–100% were estimated. Therefore, the population could be classified as susceptible (data not shown). In the last two years of the experiment (Years 4 and 5), weed-control efficacy decreased, when only HRAC-groups B and A herbicides were used compared to herbicide strategy rotating MOA every year. The weed population in HS3 shifted from a sensitive to a resistant population, when exclusively herbicides with the same MOA (B and A) were applied. During the first three years, however, efficacy of herbicides in HS3 remained very high. Gressel and Segel [21] came to similar results for maize and triazine-resistant weeds. Neve and Powles [22] selected for resistant *Lolium rigidum* plants with repeated low-dose applications of ACCase-inhibitors over five generations. With the resistance coming up in HS3, densities of *A. myosuroides* increased very fast, especially in CR1 with only winter-annual crops (Table 3). Hicks et al. [18] also reported the possibility of a fast resistance development and could show a significant correlation of resistance development and high *A. myosuroides* densities. This is also consistent with our results, which showed that the more winter-annual crops used inside the crop rotation, the higher the proportion of resistant plants.

This experiment clearly underlines the need for combining different weed-control methods. Rotating winter- and summer-annual crops effectively suppresses weed species that predominantly germinate in spring or autumn such as *A. myosuroides* [9,17]. The lower densities lead to fewer mutations—which naturally occur in a population—and therefore to a lower selection by herbicides. In addition, various crops also allow a wider range of active ingredients to be used. Moreover, if farmers avoid the repeated use of the same active ingredients for as long as possible, a development of resistance can be prevented, although this may lead to temporarily higher densities, especially when using weaker or weather-dependent active ingredients. However, the higher infestation often has no negative effect on the yield, as our results, and the results of Hicks et al. [18] have demonstrated. Hicks et al. [18] could detect yield losses between 2–12%. Nevertheless, significant losses occurred only at high and very high densities.

We are aware that the results of this experiment are limited. Geographic differences such as soil type or climate could influence the results shown. Furthermore, the effect of crop and year, which we were not able to distinguish, may have led to fluctuations in *A. myosuroides* density due to weather conditions. Nevertheless, we believe that such accurate long-term studies are important and rare. Studies like this show clear trends from which working management methods can be derived. In the near future, no new active ingredients are expected. An increasing number of active ingredients lost or might lose their application permission during the re-registration process [23]. Therefore, the available active ingredients must be used sustainably and maintained for as long as possible. Only in this way can consistent yields be guaranteed in the future.

However, autumn and spring cropping is restricted to areas with moderate temperatures during the winter. In many parts of the world, only spring cropping is possible. For those areas, several other preventive methods of non-chemical weed control are possible, such as false seedbed

preparation, rotational ploughing, intercropping, delayed sowing, higher seed rates and competitive crop cultivars [9,16,24].

Author Contributions: R.G. and Y.I.K. conceived and designed the experiments; A.K.Z. and Y.I.K. performed the experiments and analyzed the data; A.K.Z. contributed to the materials and analysis tools; R.G. and A.K.Z. wrote the paper.

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