

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

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Abstract: Cinmethylin is a potential new pre-emergence herbicide in Europe inhibiting the fatty acid thioesterases in the plastid against *Alopecurus myosuroides* 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 alone and combined with post-emergence herbicides against *A. myosuroides* and yield response of winter wheat and winter triticale. In four experiments, the effect of early and late sowing of winter cereals was included as the second factor in the experiment to investigate if late sowing can reduce *A. myosuroides* density weed control efficacy. All fields were heavily infested with *A. myosuroides* with average densities of 110–730 plants/m². Late sowing reduced 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 over all experiments. In three out of 5 experiments, grain yields were significantly increased by the herbicide treatments. The results demonstrate that cinmethylin increases the options for controlling *A. myosuroides* in winter cereals. However, it needs to be combined with other control tactics.

Keywords: black-grass; acetolactate synthase-inhibitors; acetyl CoA carboxylase inhibitors; very long chain fatty acids inhibitors; integrated weed management; seeding time

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 (Moss 1990, Melander 1995, Lutman et al. 2013, Moss 2017). *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/m² (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 (Drobny et al. 2006, Délye et al. 2007, Neve 2007, Heap 2014). 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 (Menne and Hogrefe 2012, Heap 2014). 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* (Menne and Hogrefe 2012, Dücker et al. 2019). The 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

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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 and Clarke 1994, Lutman et al. 2013). Late sowing of winter wheat in October and November significantly reduced *A. myosuroides* emergence (Gerhards et al. 2016, Menegat and Nilsson 2019) 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.

MATERIAL AND METHODS

Experimental sites. Five field experiments were conducted in winter wheat and winter triticale in 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 1. The average temperatures were higher than the long-term average in all three years. The autumns in 2018 and 2019 were extremely dry. The 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.

Experimental design. The experiments at Ihinger Hof and Entringen were realised as a two factorial randomised complete block design with three repetitions.

Table 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	–	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)

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Table 2. Experimental details of the field experiments

No. of experiment	Location	Crop (cultivar)	Year	Sowing date	Seeding rate (seeds/m ²)
1	Ihinger Hof	winter-wheat (cv. RGT-Reform)	2018	19. 10. 2017 04. 12. 2017	300 350
2	Ihinger Hof	winter-triticales (cv. Tulus)	2019	25. 09. 2018 25. 10. 2018	250 350
3	Entringen	winter-triticales (cv. Tulus)	2019	08. 10. 2018 10. 11. 2018	250 350
4	Hirrlingen	winter-wheat (cv. RGT-Reform)	2019	06. 10. 2018	400
5	Ihinger Hof	winter-wheat (cv. Patras)	2020	08. 10. 2019 31. 10. 2019	250 380

Table 3. Herbicide treatments tested in the five field experiments

Treatment	Active ingredient	Location	Application rate	Year	Crop	Time of application (DAS)
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 triticales	140
Avoxa (PP)	33.3 g/L pinoxaden, 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 (PFC)	68.3 g/kg pyroxsulam, 22.8 g/kg florasulam, 68.3 g/kg cloquintocet-mexyl	Hirrlingen	220 g/ha	2019	winter wheat	140
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 triticales	5
Pontos (FP)	240 g/L flufenacet, 100 g/L picolinafen	Ihinger Hof, Entringen, Hirrlingen	1 L/ha	2018 2019 2020	winter wheat, winter triticales	5
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 triticales	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 triticales	5

DAS – days after sowing

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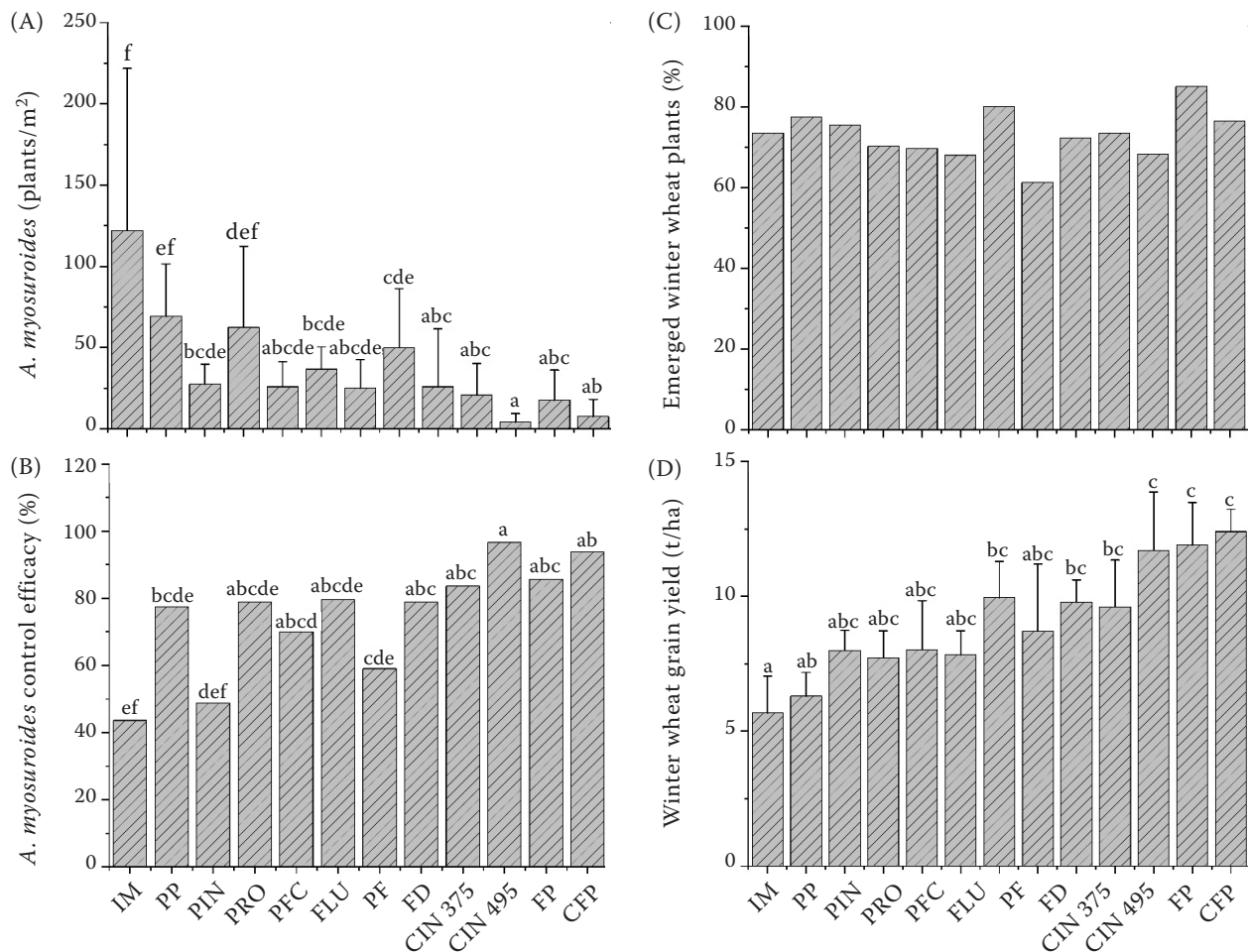


Figure 1. (A) *Alopecurus myosuroides* density/m²; (B) *A. myosuroides* control efficacy (%); (C) emerged winter wheat plants (%) according to seeding rate and (D) winter wheat grain yield (t/ha) recorded at Hirrlingen 2019. Means with the same letter are not significantly different according to Tukey HSD test at $\alpha \leq 0.05$. CON – untreated control; IM – iodosulfuron + mesosulfuron; PP – pinoxaden + pyroxsulam; PIN – pinoxaden; PRO – pro-sulfocarb; 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

The first factor was the weed control method, including 6 herbicide treatments and untreated control. The second factor was the sowing time of winter cereals, including an early and late date. The experiment at Hirrlingen was a randomised complete block design with three replications. It contained one factor, including 12 herbicide treatments and untreated control. The plot size in all experiments was 3 m × 12 m.

The herbicides tested and times of application are presented in Table 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, afterward soil moisture was increased by sufficient rainfalls. Broadleaved weed species were controlled in all plots with synthetic auxins in spring. Other pesticides and fertilisers were applied according to good professional practice.

Assessments. The density of *A. myosuroides* was determined 45, 120 and 180 DAS. In the figures, data of the second counting of *A. myosuroides* are presented. The density of cereal plants was deter-

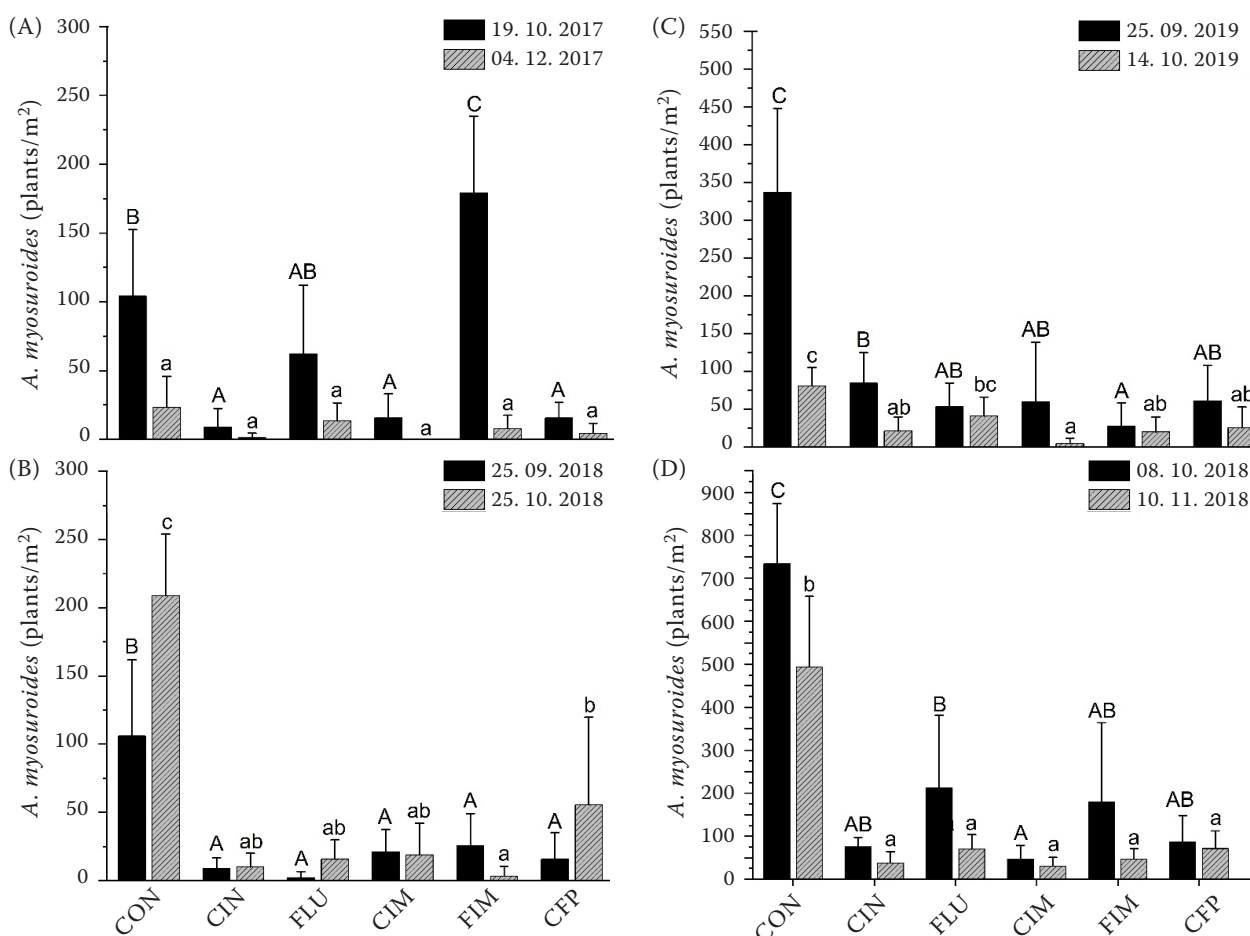


Figure 2. *Alopecurus myosuroides* density/m² recorded at (A, B, C) Ihinger Hof and (D) Entringen in April 2018–2020. Black bars represent the early seeding date, and grey bars the late seeding date. The date of seeding per year is included in the upper right corner. Means with different capital letters show significant differences within the early seeding date according to the Tukey HSD test at $\alpha \leq 0.05$. Means with different small letters show significant differences within the late seeding date according to the 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

mined recorded 28 days after herbicide application. Plants were counted in a 0.1 m² frame placed four times in each plot. Grain yield was measured in a 1.5 m × 12 m strip in each plot with a plot harvester (Wintersteiger, Elite 3, Ried im Innkreis, Austria). Grain yields were transformed into homogenous moisture of 14%.

Statistical analysis. For data analysis, the statistical software R (version 3.6.2, RStudio Team, Boston, 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 homogenise variances and to normalise 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 honestly significant difference (HSD)-test at a significance level of $\alpha \leq 0.05$.

RESULTS

Herbicide test at Hirrlingen. In the winter wheat experiment at Hirrlingen 2019, the highest density of 122 plants/m² was recorded in the CON, followed by the post-emergence combination of IM with 69 plants/m² and PIN with 63 plants/m². The lowest density of four *A. myosuroides* plants/m² was counted in the treatment within the recommended field rate of cinmethylin

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(CIN 495), followed by the combination of CFP with 7 plants/m² (Figure 1A). The 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 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 1C). The grain yield of winter wheat was lowest in the CON with 5.7 t/ha. The 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 1D).

***A. myosuroides* density at the experimental sites Ihinger Hof and Entringen.** In all four experiments, infestation rates with *A. myosuroides* were high with densities of more than 100 plants/m² in the early sown control plots. Late seeding of winter cereals reduced *A. myosuroides* densities except for the 2018 experiment at Ihinger Hof (Figure 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 2A) and the late seeding treatments of FLU at Ihinger Hof in 2019 (Figure 2C). None of the herbicide treatments provided better weed control efficacies than all other treatments consistently over all experiments.

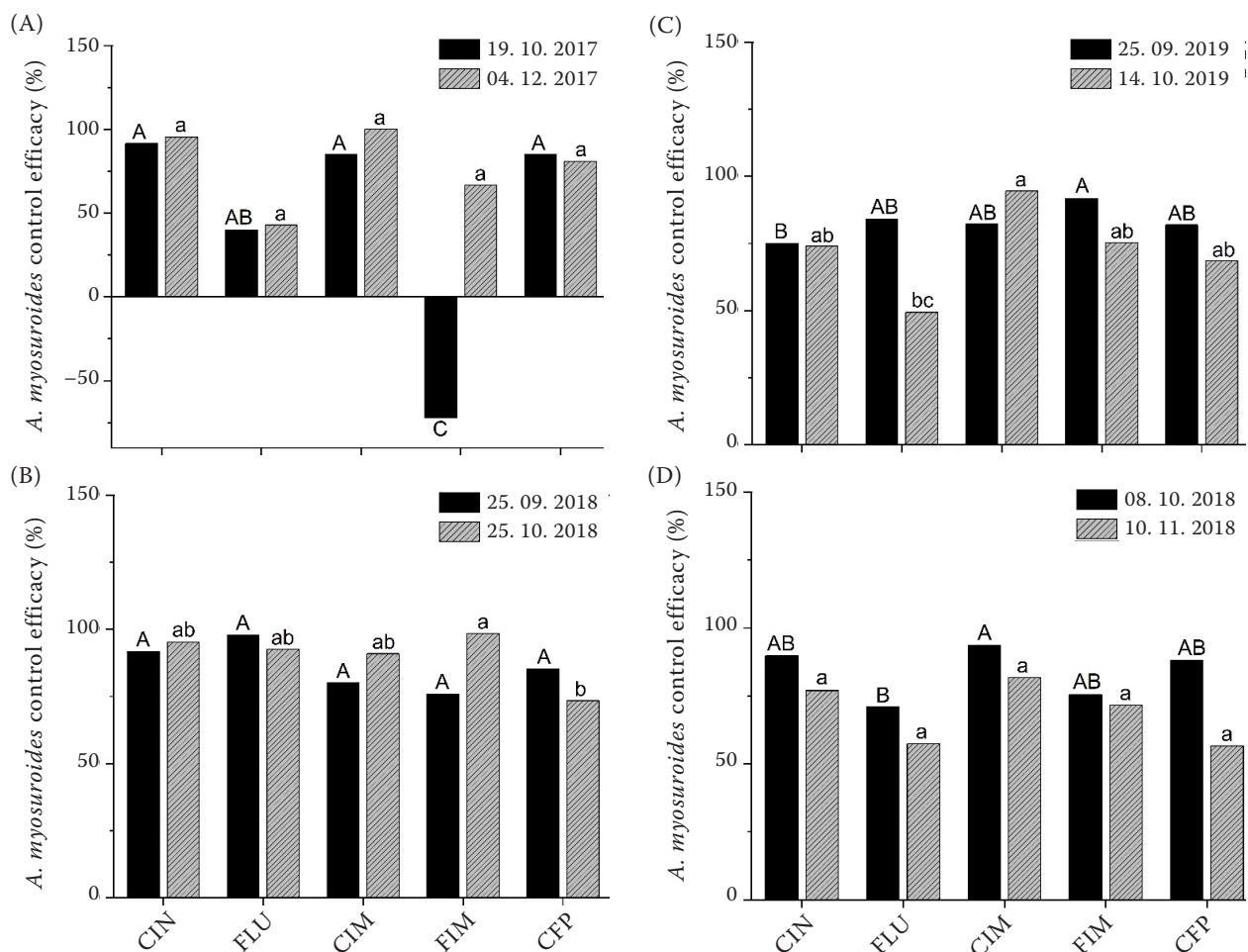


Figure 3. *Alopecurus myosuroides* control efficacy (%) recorded at (A, B, C) Ihinger Hof and (D) Entringen in April 2018–2020. Black bars show the early seeding date, while grey bars the late seeding date. The date of seeding per year is included in the upper right corner. Means with different capital letters show significant differences within the early seeding date according to the Tukey HSD test at $\alpha \leq 0.05$. Means with different small letters show significant differences within the late seeding date according to the 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

***A. myosuroides* control efficacy at Ihinger Hof and Entringen.** In three out of the four experiments (Figure 3A, B, D), 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.

Emerg ed cereal plants at the experimental sites Ihinger Hof and Entringen. Percentages of emerg ed 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 emerg ed cereal plants ranged between 25.8–22.8% for the early sown treatments and 13.7–21.9% for the late sown treatments. None of the herbicide treatments consistently reduced cereal plants.

Grain yield of winter wheat and triticale at Ihinger Hof and Entringen. Grain yield of winter cereals was 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 (Table 4).

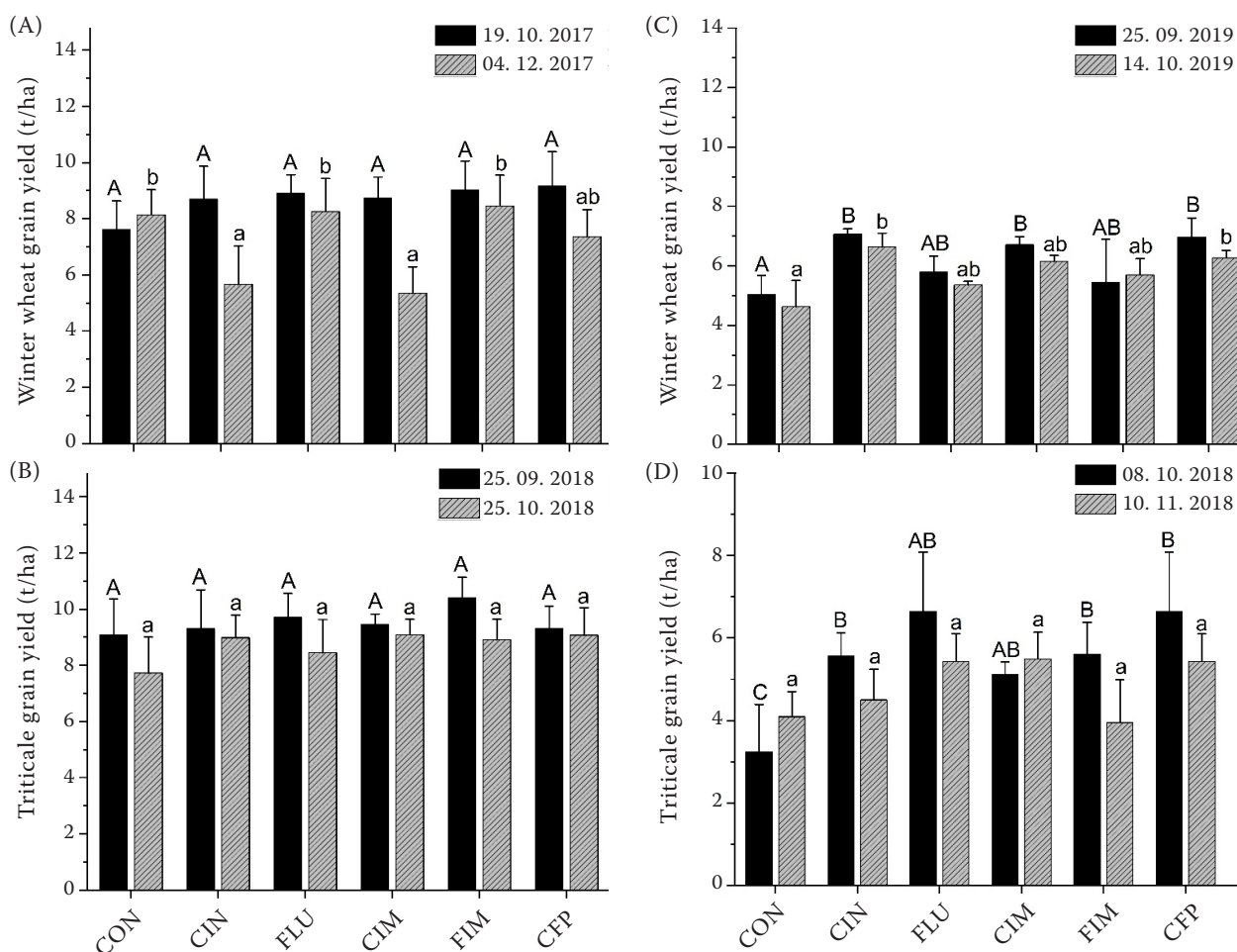


Figure 4. Winter wheat grain yield (t/ha) recorded at (A, B, C) Ihinger Hof and (D) Entringen in 2018–2020. Black bars show the early seeding date, while grey bars the late seeding date. The date of seeding per year is included in the upper right corner. Means with different capital letters show significant differences within the early seeding date according to the Tukey *HSD* test at $\alpha \leq 0.05$. Means with different small letters show significant differences within the late seeding date according to the 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

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DISCUSSION

Cinmethylin controlled more than 90% of *A. myosuroides* plants when averaged over the five field experiments. The 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-emergence 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. (2012) 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 the emergence of *A. myosuroides* from autumn until spring can explain the difference in weed control efficacy of pre-emergence herbicides (Kudsk and Kristensen 1992). Furthermore, pre-emergence herbicides can be lost due to surface evaporation or leaching in wet soils (Hammerton 1967, Kudsk and Kristensen 1992). Due to limited selectivity, the pre-emergence of such herbicides also causes a risk of crop damage when they are applied under unfavourable 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), and Menegat and Nilsson (2019). Seeds of *A. myosuroides* have the highest germination rate in September in Western European growing conditions (Moss 2017).

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

Table 4. Emerged cereal plants (%) from 2018–2020 of four experiments. Percentages of emerged cereal plants were calculated according to the corresponding seeding rate

Treatment	Sowing date	Ihinger Hof 2018	Ihinger Hof 2019	Entringen 2019	Ihinger Hof 2020
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

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

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, Schappert et al. 2018, Travlos et al. 2020).

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. The 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.

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