Impact of the safener Mefenpyr-diethyl on herbicide resistance evolution in *Alopecurus myosuroides* (Huds.) biotypes

Einfluss des Safeners Mefenpyr-diethyl auf die Entwicklung von Herbizidresistenz bei Alopecurus myosuroides *(Huds.)-Biotypen*

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Abstract

The evolution of herbicide resistance is an important topic in plant protection and agricultural practice. Safeners are commonly used in herbicides to protect crops against herbicidal damage. Although no effect on the weed control is expected, it has been theorized that the rate of evolution of non-target site resistance (NTSR) in weeds in cereals may be enhanced by use of herbicide products containing safeners. One of the most important safeners in cereals is mefenpyr-diethyl. Therefore, the possible influence of mefenpyr on herbicide resistance was studied in cooperative trials between Bayer CropScience (BCS, F-Höchst) and FH Bingen. The trials tested in parallel different herbicide resistant black-grass (*Alopecurus myosuroides* (Huds.)) biotypes under greenhouse conditions. The biotypes where chosen due to known NTSR against Atlantis WG® (4 highly resistant and 5 moderately resistant) as well as two susceptible biotypes. The populations were treated with the following three herbicide/safener regimes in six concentrations adjusted according to the anticipated biotype resistance levels. (1) mesosulfuron + iodosulfuron + without safener formulation, (2) mesosulfuron + iodosulfuron + constant mefenpyr concentration (45g/ha), (3) mesosulfuron + iodosulfuron + varying mefenpyr concentrations (ratio 5:1:15). The treatments were applied in post-emergence based on mesosulfuron to iodosulfuron ratios in Atlantis WG® (5:1). The trials were assessed visually (% effect) and by fresh weight. Dose-response curves were performed and ED_{50} values for each treatment and biotype were calculated. Results showed a varying effect of safeners which was in the most cases negligible. Depending on the biotypes mostly no impact on the safener was found for herbicide resistance. In conclusion, the trials from Bingen and F-Höchst gave evidence, that there is no significant and consistent influence of the safener mefenpyr on evolution of NTSR black-grass.

Keywords: ALS-inhibitor, black-grass, non-target-site resistance, safener

Zusammenfassung

Eines der wichtigsten Themen im Bereich Pflanzenschutz im Ackerbau ist die Entwicklung von Herbizidresistenzen in Unkräutern. Safener werden gewöhnlich in Herbiziden verwendet, um die Kulturpflanze vor möglichen Schäden zu schützen, ohne die Wirksamkeit gegenüber den Unkräutern zu verringern. Allerdings besteht die Überlegung, dass die Verwendung von Herbiziden mit Safenern auch bei Ungräsern zu einer Veränderung des Herbizidabbaus führt und die Entwicklung von nicht-Zielortresistenzen (NTSR) begünstigen könnten. Da einer der wichtigsten Safener in Getreideherbiziden Mefenpyr-diethyl ist, wurde in der vorliegenden Studie der mögliche Einfluss von Mefenpyr auf die Herbizidresistenz bei Acker-Fuchsschwanz in einem Kooperationsversuch zwischen Bayer CropScience (BCS, F-Höchst) und der FH Bingen untersucht. Es wurden parallel an beiden Standorten Acker-Fuchsschwanzherkünfte (*Alopecurus myosuroides* (Huds.)) mit unterschiedlichen Herbizidresistenzen im Gewächshaus getestet. Die Auswahl der Herkünfte erfolgte anhand bekannter Resistenzen (NTSR) gegen Atlantis WG® (4 stark resistente und 5 mittel resistente, sowie zwei sensitive Herkünfte). Es wurden drei verschiedene Herbizid-Safener Varianten in jeweils 6 Dosierungen, die an das Resistenzlevel der Herkünfte angepasst waren, getestet: (1) Mesosulfuron + Iodosulfuron + ohne Safener Formulierung, (2) Mesosulfuron + Iodosulfuron + konstant 45 g/ha Mefenpyr, (3) Mesosulfuron + Iodosulfuron + abgestufte Mefenpyr Konzentration im Verhältnis 5:1:15. Die Behandlungen wurden im Nachauflauf appliziert und basierten auf einem Mesosulfuron + Iodosulfuron Verhältnis von 5:1 (Atlantis WG®). Die Versuche wurden visuell (% Wirkung) und anhand des Frischgewichts bonitiert. Dosis-Wirkungskurven und ED_{50}-Werte wurden für jede Behandlung und jede Herkunft berechnet. Die Ergebnisse zeigten einen variierenden Einfluss des Safeners auf die Herbizidresistenz der von der Acker-Fuchsschwanzherkunft abhing. In den meisten Fällen erwies sich der Safener Einfluss als nicht groß. Somit konnten die Versuche in F-Höchst und Bingen zeigen, dass kein signifikanter und übergreifender Einfluss des Safeners Mefenpyr auf die Nicht-Zielortresistenz bei Acker-Fuchsschwanz besteht.

Stichwörter: Acker-Fuchsschwanz, ALS-Hemmer, Nicht-Zielortresistenz (NTSR), Safener

Introduction

Selective grass weed control in cereals has become more and more difficult today, because enhanced metabolic resistance (EMR) often reduce the efficacy of the herbicides. However, EMR seems to be controlled by many genes (Preston, 2003; Yuan et al., 2007; Délye et al., 2010, 2013; Petit et al., 2010; Powles and Yu, 2010). Different gene combinations of different biotypes within a field (or on neighboring fields) may result in a stronger resistant weed generation (Mencharie et al., 2006, 2007). As a consequence, weed resistance may affect more herbicides and resistance factors may be higher. Modern selective herbicides often contain a safener to ensure selectivity to the crop (e.g. mefenpyr in Atlantis WG®). These safeners increase the ability of the crop to degrade the herbicide more quickly due to activation of responsible enzymes such as CYTP450 and GSTs. Safeners can only be used if there is an effect on the crop but not on the weeds. However, in some grass weeds like *Alopecurus myosuroides* (Huds.) there are biotypes which are able to >detoxify active ingredients of herbicide by similar enzymes which are addressed by the safeners in the crop (Cummins et al., 1997; Cole, 1997; Hatzios and Burgos, 2004). This leads to the question, of whether safeners can also induce some enzymes in the weed which are responsible for EMR. If this is the case, use of safeners could increase the speed of herbicide resistance evolution. In general safeners are not responsible for evolution of EMR because EMR is an older phenomenon than use of safeners in selective weed control in cereals. However there might be an additional effect by using some safeners in some weed species or biotypes thereof. To detect possible effects of safeners on the evolution of herbicide resistance in *A. myosuroides* a cooperative greenhouse trial was conducted. The aim was to test in parallel different herbicide resistant *A. myosuroides* biotypes under greenhouse conditions in Bingen (FH-Bingen) and at the same time in Frankfurt-Höchst (Bayer CropScience). Three different herbicide treatments of mesosulfuron + iodosulfuron with and without safener mefenpyr were used to address the question, of whether safeners change dose-response curves of herbicides in EMR black-grass biotypes.

Materials and Methods

Dose-response curves of three different herbicide treatments of mesosulfuron + iodosulfuron and mefenpyr, and eleven *A. myosuroides* biotypes were performed in the greenhouses in Bingen and Frankfurt-Höchst (F-Höchst). Five replications with 5 plants per pot were performed in Bingen. In F-Höchst a defined seed density was sown per pot with five replications. The first treatment was a constant portion of blank formulation (without safener) and herbicide, the second treatment was a constant concentration of mefenpyr independent from the herbicide concentration and the third treatment was a varied concentration of safener mefenpyr. Six herbicide dosages per treatment and an untreated control were analysed. The herbicide treatments and rates and the characterization of the biotypes are given in Table 1.

Tab. 1 Herbicide treatments and *A. myosuroides* biotype characterization for the safener trial in Bingen and F-Höchst 2015 (each dose was applied with 1.0 l/ha Biopower as additive).

***Tab. 1** Herbizidbehandlungen und Acker-Fuchsschwanz Biotyp Charakterisierung des Safener Versuchs in Bingen und F-Höchst 2015 (jede Dosierung wurde mit 1,0 l/ha Biopower appliziert).*

Treat-ment	Meso-sulfuron g/ha	Iodo-sulfuron g/ha	Mefenpyr g/ha (1) blank*	(2) const.	(3) varied	senH[1]	senA[1]	DEU12053[2]	GBR 13010[2]	Elbe[2]	710[2]	601[3]	A12_508[3]	A12_522[3]	A14_616[3]	A14_575[3]
T1	0.59	0.12	0	45	1.76	x	x									
T2	1.17	0.23	0	45	3.52	x	x					x	x	x	x	x
T3	2.34	0.47	0	45	7.03	x	x									
T4	4.69	0.94	0	45	14.06	x	x	x	x	x	x	x	x	x	x	x
T5	9.38	1.88	0	45	28.13	x	x	x	x	x	x	x	x	x	x	x
T6	18.75	3.75	0	45	56.25	x	x	x	x	x	x	x	x	x	x	x
T7	37.5	7.5	0	45	112.5			x	x	x	x	x	x	x	x	x
T8	75.0	15.0	0	45	225.0			x	x	x	x	x	x	x	x	x
T9	150.0	30.0	0	45	450.0			x	x	x	x					
control	none	none	none	none	none	x	x	x	x	x	x	x	x	x	x	x

*Biotype characterisation 1=senstive, 2=high resistance factor,3=moderate resistance factor; *blank formulation without mefenpyr*

None of the biotypes showed target-site resistance (TSR) towards ALS inhibitors but decreased sensitivity towards Atlantis WG®. Biotype "Elbe" showed in previous studies a resistance factor of 19.1, biotype "710" of 6.0, indicating high resistance against Atlantis WG®. Biotype "601" showed a resistance factor of 2.6. The other biotypes were not previously characterized by dose-response curves. Results of monitoring trials showed herbicide efficacies between 10 and 55% for the biotypes "A12_508", "A12_522", "A14_616" and "A14_575". Additional biotypes were offered from Bayer Crop Science ("DEU_12053" and "GBR_13010") previously shown to have high resistance factors (>10).

Herbicide application was done with a precision lab sprayer in BBCH 12 at both trial sites. Herbicide efficacies were assessed visually 21 days after application and single plant fresh weight was measured. Based on the visual assessment and the relative fresh weight dose-response curves were calculated (STREIBIG, 1988) using the statistical software of SigmaPlot (11.0). ED_{50} values for each biotype and treatment as well as the standard error and the coefficient of determination were calculated. Based on the ED_{50} mean value of the two susceptible populations (sen A and sen H) resistant factors were calculated for fresh weight data (RF=ED_{50} res/MV(ED_{50} senH; ED_{50} senA)). A two factorial ANOVA (p = 0.05) was conducted for fresh weight data used SAS procedure GLM (software package 9.11). LSD test (Tukey 0.05) was used to compare mean values of different treatments/populations.

Results

Greenhouse trial at Bingen

The ED_{50} values based on the plant fresh weight are given in Table 2 for the results from Bingen. The ED_{50} values varied between the biotypes and the treatments. They range between a minimum of 9 ("senA"-varied) and a maximum of 1794 ("Elbe"-blank). A calculation of the ED_{50} values for biotype "A14_575" was not possible for the treatment with the blank formulation or the varying concentration of mefenpyr. Biotype "710"showed the lowest ED_{50} value after the treatment with mesosulfuron + iodosulfuron + blank formulation. Biotypes "DEU_12053", "601" and "A12_616" were best controlled with the constant concentration of the safeners and all other biotypes showed highest herbicide efficacy when treated with the varying safener concentration.

Tab. 2 ED_{50} values, standard errors and coefficients of determination for each *A. myosuroides* biotype and treatment based on relative fresh weight of the trial from Bingen.

Tab. 2 *ED_{50}-Werte, Standarderror und Bestimmtheitsmaß für jeden Acker-Fuchsschwanz-Biotyp und Behandlung, basierend auf den relativen Frischgewichten am Versuchsort Bingen.*

		A. myosuroides biotypes										
BINGEN		**senH**	**senA**	**DEU12053**	**GBR13010**	**Elbe**	**710**	**601**	**A12_508**	**A12_522**	**A14_616**	**A14_575**
BLANK	**ED_{50}**	**18**	**12**	**885**	**935**	**1794**	**197**	**172**	**21**	**11**	**74**	**-**
	SE	1.9	2.3	295.1	489.8	1134.3	74.2	97.8	2.7	1.6	39.4	-
	r^2	0.80	0.71	0.62	0.24	0.21	0.56	0.35	0.90	0.90	0.28	-
CONS-TANT	**ED_{50}**	**23**	**25**	**574**	**1211**	**1143**	**487**	**133**	**23**	**13**	**32**	**797**
	SE	3.4	2.0	90.2	623.2	232.7	655.9	70.3	1.8	1.1	29.8	264.5
	r^2	0.72	0.86	0.70	0.32	0.31	0.24	0.35	0.96	0.94	0.23	0.40
VARIED	**ED_{50}**	**12**	**9**	**1689**	**426**	**660**	**270**	**207**	**11**	**9**	**44**	**-**
	SE	1.6	1.7	910.8	135.9	121.2	62.0	271.0	0.9	2.1	38.0	-
	r^2	0.77	0.72	0.48	0.44	0.52	0.72	0.11	0.95	0.75	0.18	-

There were differences between the ED_{50} values based on the visually estimated herbicide efficacies and the ED_{50} values based on the relative fresh weights (data not shown). The visual assessment did in most cases not identify those treatments where the lowest ED_{50} values where found after the fresh weight measurement. Exceptions were biotype "GBR13010", "Elbe" and "601". These results indicate that the fresh weight measurement is necessary for the correct evaluation of herbicide efficacies.

A two-factorial ANOVA, based on the relative fresh weight showed no interaction between the *A. myosuroides* biotype and the herbicide treatment (Tab. 3). Therefore, at least the results from Bingen gave evidence, that the hypothesis of a safener effect on herbicide resistance in metabolic resistant *A. myosuroides* biotypes is not true.

Tab. 3 Results of two-factorial ANOVA based on relative fresh weight (trial Bingen; treatment – without safener, constant or variable safener concentration).

Tab. 3 *Ergebnisse der zwei-faktoriellen Varianzanalyse (Biotyp x Behandlung) basierend auf den relativen Frischgewichten (Versuchsort Bingen, Behandlung ohne Safener, mit konstanten und variablen Safenerzusatz).*

Dose %	Biotype	Treatment	Biotype x treatment
0	n.s.	n.s.	n.s.
3.906	***	n.s.	n.s.
7.813	***	***	n.s.
15.63	n.s.	**	n.s.
31.25	***	n.s.	n.s.
62.5	***	n.s.	n.s.
125	***	n.s.	n.s.
250	***	n.s.	n.s.
500	***	n.s.	n.s.
1000	*	n.s.	n.s.

n.s. - not significant; *$p < 0.05$; **$p < 0.001$; *** $p < 0.0001$

Summarizing the results of Bingen, no clear effect of the safener mefenpyr was detectable on the herbicide efficacy. There were differences between the biotypes but the resistance level of the biotypes seems to have no influence.

Greenhouse trial at F-Höchst

Results of the calculation of dose-response curves based on the relative fresh weight and the corresponding ED_{50} values from F-Höchst are given in Table 4.

Tab. 4 ED_{50} values, standard errors and coefficients of determination for each *A. myosuroides* biotype and treatment based on relative fresh weight of the trial from F-Höchst.

Tab. 4 *ED_{50}-Werte, Standardabweichung und Bestimmtheitsmaß für jeden Acker-Fuchsschwanz-Biotyp und Behandlung, basierend auf den rel. Frischgewichten am Versuchsort F-Höchst.*

		Black-grass biotypes										
F-HÖCHST		**senH**	**senA**	**DEU12053**	**GBR13010**	**Elbe**	**710**	**601**	**A12_508**	**A12_522**	**A14_616**	**A14_575**
BLANK	**ED_{50}***	**16**	**15**	**324**	**86**	**469**	**108**	**42**	**9**	**8**	**8**	**14**
	SE	1.7	1.5	51.9	18.9	166.8	24.9	9.8	1.8	3.3	5.0	6.9
	r^2	0.90	0.93	0.85	0.85	0.85	0.88	0.78	0.95	0.84	0.83	0.83
CONS-TANT	**ED_{50}**	**19**	**17**	**2090**	**277**	**986**	**157**	**30**	**15**	**12**	**26**	**38**
	SE	1,9	1.8	1586.2	75.4	409.9	40.2	6.2	2.2	3.4	6.6	13.6
	r^2	0.90	0.90	0.71	75.43	0.70	0.84	0.89	0.95	0.86	0.87	0.86
VARIED	**ED_{50}**	**10**	**9**	**1224**	**251**	**1187**	**302**	**57**	**12**	**21**	**42**	**94**
	SE	1.0	0.0	419.7	76.1	543.5	107.7	10.5	2.4	4.8	10.6	34.8
	r^2	0.91	0.94	0.83	0.77	0.79	0.83	0.65	0.92	0.84	0.76	0.74

The ED_{50} values ranged between a minimum of 8 ("A14_616" – blank) and a maximum of 2090 ("DEU12053" – constant). The ED_{50} values differed between the biotypes and the treatments. For all biotypes except for the susceptible biotypes "senH" and "senA", and the resistant biotype "601" the lowest ED_{50} values were found with the blank formulation. A two-factorial ANOVA based on the relative fresh weight showed varying results (Tab. 5). Depending on the herbicide dosages interactions between the biotype and treatment were found.

Tab. 5 Results of two-factorial ANOVA based on relative fresh weight (trial F-Höchst; treatment – without safener, constant or variable safener concentration).

Tab. 5 *Ergebnisse der zwei-faktoriellen Varianzanalyse (Biotyp x Behandlung) basierend auf den relativen Frischgewichten (Versuchsort F-Höchst, Behandlung ohne Safener, mit konstanten und variablen Safenerzusatz).*

Dose %	Biotype	Treatment	Biotype x treatment
0	n.s.	n.s.	n.s.
3.906	*	***	n.s.
7.813	**	n.s.	***
15.63	n.s.	***	n.s.
31.25	***	n.s.	n.s.
62,5	***	***	n.s.
125	***	n.s.	*
250	***	*	**
500	***	**	**
1000	***	***	n.s.

n.s. - not significant; *P < 0.05; ** < 0.001; *** P < 0.0001

Comparison of both trial sites

Summarizing the results from Bingen and F-Höchst, resistance factors were calculated based on the mean value of the susceptible biotypes for each *A. myosuroides* biotype, treatment and trial site (Tab. 6).

Tab. 6 Resistant factors of *A. myosuroides* biotypes treated with different mesosulfuron/iodosulfuron concentrations without and with safener mefenpyr based on ED_{50} values.

***Tab. 6** Resistenzfaktoren der Acker-Fuchsschwanz-Biotypen behandelt mit verschiedenen Mesosulfuron/Iodosulfuron Konzentrationen mit und ohne dem Safener Mefenpyr, basierend auf den ED_{50}-Werten.*

	BINGEN			F-HÖCHST		
Biotype	**blank**	**varied**	**constant**	**blank**	**varied**	**constant**
senH	1.2	1.0	1.1	1.0	1.1	1.0
senA	0.8	1.0	0.9	1.0	0.9	1.0
DEU12053	> 15.0	> 15.0	> 15.0	> 15.0	> 15.0	> 15.0
GBR13010	> 15.0	> 15.0	> 15.0	5.5	> 15.0	> 15.0
Elbe	> 15.0	> 15.0	> 15.0	> 15.0	> 15.0	> 15.0
710	13.4	> 15.0	> 15.0	7.0	8.6	> 15.0
601	11.7	5.6	> 15.0	2.7	1.6	5.9
A12_508	1.4	1.0	1.0	0.6	0.8	1.3
A12_522	0.7	0.6	0.9	0.5	0.7	2.2
A14_616	5.0	1.3	4.3	0.5	1.4	4.3
A14_575	>>	> 15.0	>>	0.9	2.1	9.8

Discussion

The results from F-Höchst differ from the results obtained in Bingen. While the data from F-Höchst suggest that the safener mefenypr increases the ED_{50} values for the treatment 'constant' safener concentration, the data from Bingen did not show this influence so clearly. Possible reasons for the variation between the trial locations might be due to differences in, for example sowing, and application methods and growing conditions on the efficacy of herbicides. In Bingen, five pre-germinated seeds per pot were analysed whereas in F-Höchst the seed density was defined with a constant seed weight per pot. Therefore, different seed densities were given at both trial locations. The seed densities clearly influenced the plant growth and might influence the herbicide efficacy (MENNE et al., 2012). Additionally, the fresh weight measurement was different. Single plant weights were obtained in Bingen, whereas all plant from one pot were measured in F-Höchst. Another explanation for the varying results between F-Höchst and Bingen might be the trial design. In Bingen a randomized setting was performed to exclude side effects as far as possible. In F-Höchst the pots were placed in blocks without randomization, clustering all pots treated with mesosulfuron + iodosulfuron + blank formulation, mesosulfuron + iodosulfuron + varied safener concentration, and mesosulfuron + iodosulfuron + constant safener concentration. It seems to be possible, that the cluster treated with the blank formulation had better conditions for herbicide efficacy.

The visual assessment of the herbicide efficacy resulted in different results between Bingen and F-Höchst. The environmental influences on herbicide efficacy are bigger than suggested. However, the trials from Bingen and F-Höchst gave evidence, that there is no significant and consistent influence of the safener mefenpyr on NTSR in *A. myosuroides*.

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