Impact of multiple resistance mechanisms in black-grass (*Alopecurus myosuroides* Huds.) populations on the activity of cereal herbicides

Einfluss von multiplen Resistenzmechanismen in Ackerfuchsschwanz (Alopecurus myosuroides Huds.)-Populationen auf die Wirksamkeit von Getreideherbiziden

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Summary

Herbicide resistance to different modes of action is spreading not only in the intensive winter wheat producing areas of Northern Germany, but also in other regions. In this investigation, four selected biotypes of black-grass (*Alopecurus myosuroides* Huds.) from Lower Saxony, Schleswig-Holstein and Baden-Württemberg were tested for resistance to different herbicides in greenhouse monitoring trials. Using the Pyrosequencing™ technology, leaf material of the different biotypes was screened for ACCase and ALS target-site mutations. The resistance mechanism profiles of the tested biotypes were first completed with metabolism studies of selected herbicides. Finally, the effectiveness of different ACCase and ALS herbicides was investigated in dose response studies under controlled conditions. The results of the analysis showed the occurrence of multiple resistance mechanisms – target-site and non-target site – in the different black-grass biotypes. The calculated resistance factors for the herbicides varied between biotypes and occurring resistance mechanisms. Independent of the range of the resistance factors, low levels already caused decreased effectiveness under real field conditions. Despite confirmed target-site and enhanced metabolic resistances, most of the tested herbicide mixtures and sequence applications provided useful efficacy levels. Soil-acting herbicides have become the backbone for controlling heavy infestations of black-grass populations in practice. Especially flufenacet and its mixtures provide high efficacy levels for the reduction of the first grass weed flush. Within the herbicide strategy study, Atlantis WG® appeared as the most effective post emergence applied product, especially on biotypes with metabolic resistances. With regard to missing alternative solutions in the near future, a mid to long-term sustainable crop-production can only be assured with mixtures and sequence applications of different herbicides and modes of action combined with integrated weed management tools.

Keywords: ACCase inhibitors, ALS inhibitors, enhanced metabolic resistance, herbicide management strategies, target-site resistance

Zusammenfassung

Herbizidresistenzen sind nicht mehr nur ein Problem der Winterweizenanbaugebiete in Norddeutschland, sondern kommen vermehrt auch in anderen Getreideanbaugebieten in Deutschland vor. In den vorliegenden Untersuchungen wurden vier ausgesuchte Ackerfuchsschwanzherkünfte aus Niedersachsen, Schleswig-Holstein und Baden-Württemberg auf Herbizidresistenz gegenüber verschiedenen Herbiziden in Gewächshausversuchen getestet. Die Ungrassamen und das Pflanzenmaterial wurden mittels Pyrosequencing™ auf ACCase und ALS Target-Site Mutationen untersucht. Das Resistenzprofil der Herkünfte wurde zusätzlich mit Metabolismusstudien zu ausgesuchten Wirkstoffen vervollständigt. Schließlich wurde die Wirksamkeit der Herbizide in Dosis-Wirkungsuntersuchungen unter kontrollierten Bedingungen überprüft. Die Ergebnisse der Analysen zeigten das Vorkommen von multiplen Resistenzmechanismen, sowohl Target-Site als auch Nicht-Target-Site Resistenzmechanismen, in allen untersuchten Herkünften. Die berechneten Resistenzfaktoren für die einzelnen Herbizide variierten zwischen den Herkünften und den nachgewiesenen Resistenzmechanismen. Unter realen Feldbedingungen wurde bereits mit niedrigen Resistenzfaktoren eine unzureichende Wirkung erzielt. Trotzdem konnten durch Mischungen und Sequenzapplikationen von unterschiedlichen grasaktiven Produkten hohe Wirkungsgrade erzielt werden. Bodenwirksame Herbizide sind ein wichtiger Bestandteil der Herbizidmanagementmaßnahmen geworden. Besonders Flufenacet und dessen Mischungsprodukte haben sich als sehr wirksame Bodenwirkstoffe zur Bekämpfung der ersten Auflaufwelle von Ackerfuchsschwanz herausgestellt. In den Untersuchungen zur Herbizidstrategie erwies sich Atlantis WG®, besonders auf Biotypen mit metabolischer Resistenz, als wirkungsstärkstes Nachauflaufherbizid. Dennoch kann eine mittel- bis langfristig nachhaltige Pflanzenproduktion, mit Hinsicht auf kurzfristig fehlende alternative Lösungen, nur durch eine integrierte Anwendung von Mischungen und Sequenzapplikation von unterschiedlichen Herbiziden und Maßnahmen der Produktionstechnik erfolgreich sein.

Stichwörter: ACCase Inhibitoren, ALS Inhibitoren, Herbizidmanagement Strategien, metabolische Resistenz, target-site Resistenz

1. Introduction

Herbicide resistance in black-grass (*Alopecurus myosuroides* Huds.) has become a common threat in the agricultural areas of Germany. The selection and distribution has taken place slower than in other European countries like France and UK in the last decades (MOSS, 1987; CHAUVEL and GAZQUEZ, 1990; HEAP, 2011). Changes in production systems such as dominance of winter crops with mono-cropping of winter wheat, increase of minimum tillage practices, early sowing dates as well as economic pressure due to low commodity prices and limited herbicide options, have caused a tremendous selection pressure within weed populations in the last years. More and more locations are found with decreased sensitivity to different compounds and site of action classes. Control options become more and more limited because of loss of compounds due to European regulations and a lack of new innovations by the industries. Short to mid-term, farmers have to manage the weed infestation with the existing and available management tools. In most cases, management decisions are taken by experience and cost pressure, without exact knowledge of the sensitivity properties of the weed populations. Monitoring investigations are only conducted in cases of farmers` complaints about product effectiveness, and therefore, in cases of positive confirmation, it is as a rule too late for preventive measures. Ring tests have shown that target-site resistances are easier to identify than non-target-site resistance mechanisms (PETERSEN et al., 2010). But it is assumed that non-target-site resistance mechanisms are the majority of herbicide resistances in farmers' fields. Against this background, there is some speculation if that is really the case. Random monitoring has not been carried out so far with a detailed analysis of possible resistance mechanisms. With knowledge of findings by DELYE et al. (2007) and HESS et al. (2012), the occurrence of target-site resistance mechanism is often underestimated. In case of a general approval of this assumption, it must be questioned why there have not been more farmer complaints. It can be assumed that in most cases the number of surviving plants can be neglected. However, the selection process is ongoing. The identification of single resistance mechanism in a weed population is insufficient for giving helpful management advice to farmers. Only a comprehensive diagnosis of a population regarding target-site and non-target-site resistance mechanism can provide useful information for an advanced management recommendation. Such comparable studies have not been conducted before. In 2010, selected *A. myosuroides* biotypes were extensively investigated regarding involved resistance mechanisms and their influence on the activity of commonly used herbicides. Additional outdoor studies should demonstrate the effectiveness of herbicide management strategies for the control of these resistant *A. myosuroides* biotypes.

2. Materials and methods

2.1 Selection of seed samples and bioassays

In monitoring studies, four *A. myosuroides* biotypes were identified with suspected lower sensitivity to most of the tested herbicides (Tab. 1). Dose response studies with seeds of all four locations were conducted under controlled glasshouse conditions.

The seed samples were cleaned, pre-germinated and sown in 8 cm pots (Fa. Jiffy) filled with a standard field soil (loamy silt) with four repetitions each. The pots were placed in a glasshouse with 60 % humidity, 22 °C day, 15 °C night and 12 h light with minimum 2200 µE/m^{2}s (at 555 nm; sodium high pressure lamps if necessary). After germination of the seeds the plants were thinned to 7 plants per pot. Five sensitive reference biotypes were used for verification. Five herbicides [Ralon Super® (fenoxaprop-p-ethyl + mefenpyr-diethyl) + FHS (adjuvant), Axial 50® (pinoxaden + cloquintocet-mexyl), Atlantis WG® (mesosulfuron-methyl + iodosulfuron-methyl + mefenpyr-diethyl) + FHS, Lexus 50 DF® (flupyrsulfuron-methyl) + Trend 90 and Broadway® (pyroxsulam + florasulam + cloquintocet-mexyl) + Broadway FHS of two different modes of action] were applied at growth stage BBCH 12-21 with a standard laboratory track sprayer (teejet nozzle XR8002, pressure 2.4 bar, water amount 300 l/ha). All products were sprayed with seven different dose rates for dose response analysis. The evaluation was done three weeks after application by visual assessment of the damages (%) in comparison with the untreated control.

Tab. 1 Characterisation of herbicide resistance levels in different black-grass biotypes – results from previous monitoring studies.

Tab. 1 *Einstufung der verschiedenen Ackerfuchsschwanzbiotypen in Herbizidresistenzklassen – Ergebnisse aus vorangegangenen Monitoringstudien.*

		biotype 1	biotype 2	biotype 3	biotype 4	resistant standard	sensitive standard
ACCase	Ralon Super	RRR	RRR	RRR	RR	RR	S
	Topik EC100	RRR	RRR	RRR	RR	RR	S
	Axial 50	RRR	RRR	RRR	RR	RR	S
	Traxos 50	RRR	RRR	RRR	RR	RR	S
	Focus ultra	RRR	S	RRR	S	S	S
ALS	Atlantis WG	RR	RR	S	RRR	S	S
	Lexus 50 DF	RRR	RR	RR	RRR	RR	S
	Broadway	RRR	RR	RR	RRR	RR	S

(The analysis of the results followed the 'R' system of MOSS et al. (1999); Topik EC100® and Focus ultra® were only tested in the monitoring)

Additionally, different herbicide mixtures and sequence application strategies were tested with their standard field dose rates under outdoor conditions (application rates see Tab. 4). Seeds of three of the tested biotypes were sown in 16 cm plastic pots (Fa. Pöppelmann) filled with a standard field soil (loamy silt) with three repetitions each. The pots/plants were treated according to the different strategies (Tab. 4) at growth stage BBCH 10-(11), 12-21 and/or (21)-25 using a standard laboratory track sprayer (see above). Additional to the above mentioned products, the following products were used: Cadou SC® (flufenacet), Bacara Forte® (flufenact + flurtamone + diflufenican), Herold SC® (flufenacet + diflufenican), Malibu® (flufenacet + pendimethalin), Stomp Aqua® (pendimethalin), Husar OD® (iodosulfuron-methyl + mefenpyr-dietyhl) and Traxos 50® (clodinafop-propagyl + pinoxaden + cloquintocet-mexyl). The evaluation was done three weeks after application by visual assessment of the damages (%) in comparison with the untreated control.

[Atlantis WG®, Bacara Forte®, Cadou SC®, Herold SC®, Husar OD®, Ralon Super® registered products of Bayer CropScience Germany GmbH. Focus ultra® Malibu® Stomp Aqua® registered products of BASF SE. Axial®, Topik EC100® and Traxos 50 ® registered products of Syngenta Agro GmbH. Lexus 50 DF® registered product of DuPont de Nemours Germany GmbH. Broadway® registered product of Dow AgroSciences GmbH.]

2.2 Analysis of resistance alleles

In 2010, plant samples from the four selected locations were taken to determine the resistance status in the *A. myosuroides* populations (Tab. 2). Alleles of the chloroplastic ACCase gene as well as the ALS gene of *A. myosuroides* were analyzed using the Pyrosequencing™ technology (NORDSTRÖM et al., 2000) as described by WAGNER et al. (2007) and MENNE et al. (2008).

DNA was extracted from each individual plant of each biotype using a commercial DNA extraction kit. PCR products were purified and analyzed using the Pyrosequencer PSQ-96 according to the manufacturer's instructions (Pyrosequencing, Inc, Uppsula, Sweden). Results are given as mean percentage of allele frequencies. For herbicide metabolism analysis, two tillers of each plant were incubated with radio labeled fenoxaprop-p-ethyl and mesosulfuron-methyl following the description by RUIZ-SANTAELLA et al. (2010). Extracts of the plant samples were analyzed using HPLC analysis with a Ramona 92 detector (Fa. Raytest).

Tab. 2 Genotypic and physiological characterisation of herbicide resistance in different black-grass biotypes – data in % of the confirmed findings.

Tab. 2 *Genotypische und physiologische Charakterisierung der Herbizidresistenz bei verschiedenen Ackerfuchsschwanzbiotypen – Ergebnisse in % der nachgewiesenen Resistenzen.*

		biotype 1	biotype 2	biotype 3	biotype 4
ACCase	I1781L	25% heterozygote	-	57% heterozygote	-
	W2027C	-	72% heterozygote	-	-
	D2078G	25%heterozygote 25% homozygote	-	28% heterozygote	-
ALS	P197T	57% heterozygote	-	-	-
	W574L	-	-	-	38% heterozygote 13% homozygote
EMR	fenoxaprop-p-ethyl	50% intermediate	13% intermediate*	12% intermediate	50% intermediate
	mesosulfuron-methyl	88% intermediate 12% high	28% intermediate 58% high	76% intermediate 12% high	88% intermediate

(*Results from 2008; EMR = enhanced metabolism resistance)

2.3 Statistical analysis

The GNU-licensed statistic program "R" and the additional "drc" package (RITZ and STREIBIG, 2005) were used for the evaluation of the dose response results. The data were calculated with a non-linear regression and the four-, three- and two-parameter log-logistical model following KNEZEVIC et al. (2007). Further dose-response models were checked and excluded following a variance analyses (significance level $\alpha = 5$ %). For comparison of the dose response curves, the ED_{50} and ED_{90} values, as well as the resistance factors (RF), were calculated.

3. Results

3.1 Description of the selected biotypes with suspected resistance profile, bioassay and single plant analysis

All four different biotypes represent population mixtures of survivors from farmers' fields. The selected biotypes, which were suspicious regarding their sensitivity to different products in the field, showed resistance to most of the tested herbicides in the monitoring (Tab. 1). The aryloxyphenoxy-propionate (Fop) and phenylpyrazoline (Den) herbicides failed either completely (RRR) or showed only side effects (RR) on the selected biotypes. Only the cyclohexandione (Dim) compound was able to control the biotypes 2 and 4. The analysis of the resistance mechanisms (Tab. 2) demonstrated the differences between the individual biotypes. Pyrosequencing analysis detected different target-site mutations in the biotypes 1-3. Two mutations Ile-1781-Leu and Asp-2078-Gly were identified in the biotypes 1 and 3, whereas a Trp-2027-Cys mutation was found in biotype 2. The percentage of these target-site mutations was higher than 50 % in the given populations. A certain proportion of EMR (enhanced metabolism resistance) could be identified too, especially in biotype 1 with 50 % intermediate EMR for fenoxaprop-p-ethyl. In contradiction to the first three biotypes, the ACCase compounds still showed side effects on biotype 4. This biotype also contained a certain level of 50 % intermediate EMR to fenoxaprop-p-ethyl. In most cases, the ALS compounds failed completely in controlling biotypes 1 and 4 (RR-RRR). The pyrosequencing analysis detected a percentage of 57 % of the Pro-197-Thr mutation for the biotype 1 and a percentage of 51 % of the Trp-574-Leu mutation in biotype 4. The populations of these biotypes also contained a certain level of EMR (≥ 88 % intermediate) to mesosulfuron-methyl. Biotype 2 was insufficient (RR) controlled by all three ALS compounds. With 58 %, the results showed a high EMR level of mesosulfuron-methyl. In contradiction, the efficacy of Atlantis WG was still sufficient for biotype 3 despite an intermediate EMR level of 76 % for mesosulfuron-methyl. The compounds Lexus 50 DF and Broadway provided only side effects on this biotype.

3.2 Resistance profile of selected biotypes based on dose response studies

The results of the dose response studies were compared to the mean values, ED_{50} and ED_{90}, of five different sensitive biotypes. The comparison of the mean values (Fig. 1) illustrates the variability between the different "sensitive" biotypes. Highest differences were obtained for Lexus 50 DF with a range of +98 % to -47 %. Differences to the other compounds were lower. The dose response results for Ralon Super and Axial 50 varied between biotypes and the occurring resistance mechanisms. However the graduation between the biotypes was the same. The calculated resistance factors for Axial 50 were always lower as compared to Ralon Super (Tab. 3). They varied on the ED_{50} level between RF 6-12 for Axial 50 and RF 14-144 for Ralon Super. Differences in the slopes of the dose-response curves of the resistant biotypes as compared to the sensitive biotypes caused much higher resistance factors based on the ED_{90} level for both compounds (RF 19-168 for Axial 50 and RF 76->3000 for Ralon Super). The most moderate resistance factors were observed for biotype 4, where only an intermediate EMR to fenoxaprop-p-ethyl could be identified. The Trp-2027-Cys mutation in biotype 2 did not change the activity of Axial 50 very much whereas the effectivity of Ralon Super was much more influenced, and the resistance factors increased by a factor of 2 to 8. The biotypes 1 and 3 with two mutations (Ile-1781-Leu and Asp-2078-Gly) showed the highest resistance factors for the ACCase compounds (RF 7-12 and 144-168 for Axial 50, RF 88-144 and >3000 for Ralon Super). Differences in the resistance factors for the ALS inhibitors were much higher between biotypes with different resistance mechanisms (Tab. 3). However the graduation between biotypes, resistance mechanisms and compounds, were the same in most cases. Biotypes 2 and 3 with only moderate to high EMR to mesosulfuron-methyl showed the lowest resistance factors (RF 3-22 and 4-54 for Atlantis WG, 6-11 and 14-145 for Lexus 50 DF and 14-60 and 25-489 for Broadway). Target-site mutations in the ALS gene caused much higher resistance factors. The Pro-197-Thr mutation in biotype 1 increased the values to RF 101 and 514 for Atlantis WG, 429 and >3000 for Lexus 50 DF and 419 and 1562 for Broadway. The Trp-574-Gly mutation caused an insensitivity to all products. With a 50 fold application rate it was not possible to reach even a 50 % efficacy. The ED_{50} as well as ED_{90} values had to be interpolated with the statistical program. The calculated resistance factors were higher than RF >3000.

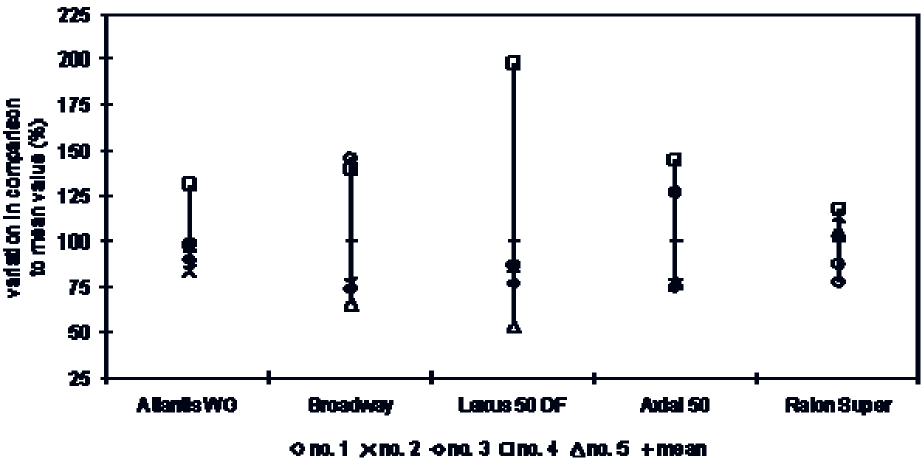

Fig. 1 Percentage variation of ED_{90} values of five different sensitive *A. myosuroides* biotypes in comparison to the calculated mean value.

Abb. 1 *Prozentualer Vergleich der ED_{90} Werte von fünf unterschiedlichen sensitiven A. myosuroides-Herkünften im Vergleich zum berechneten Mittelwert.*

Tab. 3 Resistance factors (RF) based on ED_{50} and ED_{90} values for four different *A. myosuroides* biotypes and five different herbicides.

Tab. 3 *Resistenzfaktoren (RF) auf Basis der ED_{50} und ED_{90} Werte von vier unterschiedlichen A. myosuroides-Biotypen und fünf unterschiedlichen Herbiziden.*

	biotype 1		biotype 2		biotype 3		biotype 4	
herbicide	RF ED_{50}	RF ED_{90}	RF ED_{50}	RF ED_{90}	RF ED_{50}	RF ED_{90}	RF ED_{50}	RF ED_{90}
Ralon Super	88	>3000	34	640	144	>3000	14	76
Axial 50	7	168	10	20	12	144	6	19
Atlantis WG	101	514	22	54	3	4	2908	>3000
Lexus 50 DF	429	>3000	11	145	6	14	>3000	>3000
Broadway	419	1562	60	489	14	25	>3000	>3000

3.3 Herbicide strategies for the control of selected biotypes with multiple resistance mechanisms

The effectiveness of different herbicide management strategies was tested in an outdoor pot trial.

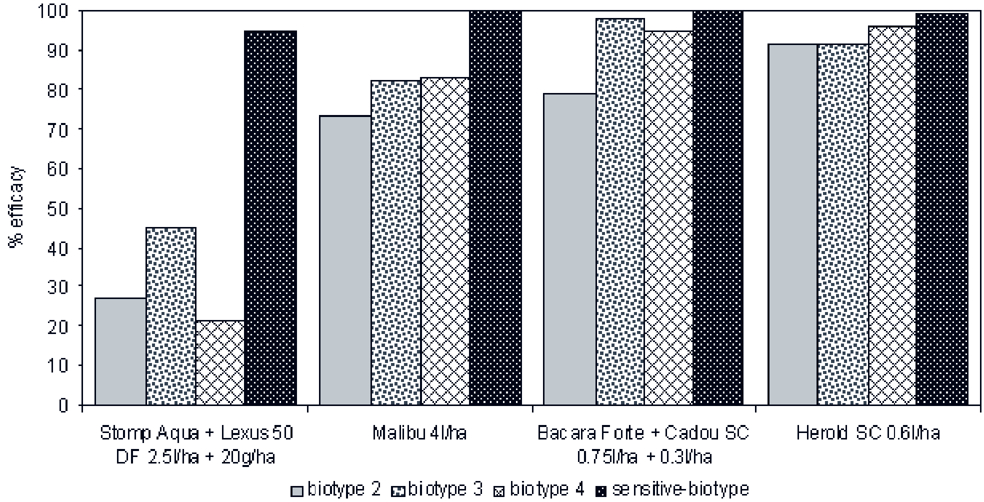

Fig. 2 Effectiveness of different soil acting herbicides on the control of *A. myosuroides* biotypes with EMR and target-site resistances to ACCase/ALS inhibitors in an outdoor pot trial.

Abb. 2 *Wirksamkeit von Bodenwirkstoffen zur Kontrolle von A. myosuroides-Biotypen mit EMR und Target-Site Resistenzen gegenüber ACCase/ALS Inhibitoren im Topfversuch unter Außenbedingungen.*

Tab. 4 Effectiveness of different weed management strategies for the control of *A. myosuroides* biotypes with EMR and target-site resistances to ACCase/ALS inhibitors

Tab. 4 *Wirksamkeit unterschiedlicher Unkrautbekämpfungsstrategien zur Kontrolle von A. myosuroides-Biotypen mit EMR und Target-Site Resistenzen gegenüber ACCase/ALS Inhibitoren*

weed management strategy		applications-time			efficacy in %				costs €/ha*
preem. applied herbicide	postem. applied herbicide				biotype 2	biotype 3	biotype 4	sensitive biotype	
Herold SC 0.6 l/ha	Atlantis WG 0.5 kg/ha	A		C	good	very good	very good	very good	119
Bacara Forte + Cadou SC 0.75 + 0.3 l/ha	Atlantis WG 0.5 kg/ha	A		C	good	very good	very good	very good	120
	Traxos 50 "fb" Atlantis WG 1.2 l/ha fb 0.5 kg/ha	A	B	C	sufficient	very good	very good	very good	163
	Atlantis WG + Hussar OD 0.5 kg/ha + 0.1 l/ha	A		C	very good	very good	good	very good	168
Malibu 4 l/ha	Atlantis WG 0.5 kg/ha	A		C	good	very good	sufficient	very good	121
	Traxos 50 "fb" Atlantis WG 1.2 l/ha fb 0.5 kg/ha	A	B	C	very good	very good	sufficient	very good	164
	Traxos 50 1.2 l/ha	A	B		sufficient	sufficient	sufficient	very good	102
Stomp Aqua + Lexus 50 DF 2.5 l/ha + 20 g/ha	Traxos 50 1.2 l/ha	A	B		weak	weak	insufficient	very good	96
	Broadway 0.22 kg/ha	A		C	weak	sufficient	weak	very good	118
	Atlantis WG 0.5 kg/ha	A		C	weak	very good	insufficient	very good	114

very good	good	sufficient	side effects	weak	insufficient
(95-100)	(90-94)	(80-89)	(70-79)	(50-69)	(<50)

(A = BBCH 10-(11); B = BBCH 12-21; C = BBCH (21)-25; Atlantis WG, Lexus 50 DF and Broadway were always applied with their corresponding adjuvants; fb = followed by; *BEISELEN, 2010)

This study could only be conducted with three biotypes due to missing germination of biotype 1 in the outdoor experiment. The leaf-acting herbicides were applied either in mixtures or in sequence with products of alternative sites of action. All sequences with flufenacet based products (Herold SC, Bacara Forte + Cadou SC and Malibu) resulted in control of roughly ≥ 80 % for all biotypes tested (Fig. 2). All strategies with Stomp Aqua + Lexus 50 DF were insufficient, especially on biotypes with target-site resistances or higher EMR (Tab. 4). The biotype 2 with high EMR and biotype 4 with ALS target-site resistance were the most difficult to control biotypes. Due to the high level of efficacy of flufenacet based products, the additional effect of the post applied product was rather low. The impact was much higher in sequence with Stomp Aqua + Lexus 50 DF. The efficacy level for biotype 2 could be doubled and the major control level for biotype 3 was obtained by Broadway or Atlantis WG. The cost calculation, based on a price list of BEISELEN (2010), reflects the cost structure of the applied product strategies. Neither cheap nor expensive single solutions ensure sufficient control of grass weeds with multiple resistance mechanisms.

4. Discussion

4.1 Resistance profile of selected biotypes and dose response studies

Most dose-response analyses which are described in scientific publications had been conducted with one or two different, so called, "sensitive" biotypes for comparison. As scientists, we can ask whether it is admissible to use only one or two biotypes. From the authors` viewpoint, concerning this question, there is no defined standard. Most researchers are dependent on external suppliers for their biotypes. The origin of these populations is unknown in most cases. An exact definition of „sensitivity", however, is not available. Therefore, the border area between natural variation of sensitivity of populations and initial „insensitivity" or possible resistance is fluid. A resistance confirmation based on a single biotest with more or less accurate dose-response analysis is not sufficient. At this point, work should be proceeded following the corresponding guideline of HEAP (2005). Within these guidelines, it is recommended that: „a better scientific view point would be that a population differs significantly in response to a herbicide when compared to the average response from numerous populations", although the exact number is not defined here. In our studies of the dose-response analysis, the mean of five different, so called, „sensitive" biotypes was calculated for comparison. The comparison clarifies the influence of variation in sensitivity of the different biotypes. Highest differences were obtained for Lexus 50 DF with a range of +98 % to -47 %. The use of either one or the other sensitive biotype would cause e.g. in biotype 2 differences in the RF values of factor 2 based on ED_{50} values and factor 4 based on ED_{90} values. The most "insensitive" sensitive biotype had a RF value of 4 (based on ED_{90}) compared to the most "sensitive" sensitive biotype.

The "quality" of the dose response curves with their calculated slopes and ED_{50} and ED_{90} values is dependent, among other things, on the accuracy of the study and the chosen dose rates. STREIBIG (2011) pointed out that a minimum of six to seven dose rates are needed for an accurate dose response analysis. This is also a general flaw of many research studies. Three dose rates each should be above and below the expected ED_{50} value. These requirements could not be met in all cases of our own studies despite previous pre-trials which had been conducted.

The results of the dose response studies for Ralon Super and Axial 50 were not directly in line with the efficacy results of the monitoring. Both herbicides failed either completely (RRR) or showed only side effects (RR) on the selected biotypes. However, the scientific viewpoint unveils essential differences between both products. As expected, the resistance factors for the compounds to biotype 4 with an intermediate level of EMR to fenoxaprop-p-ethyl had the lowest values (RF 6 and 14 for ED_{50}). The different occurring target-site mutations influenced the efficacy of Ralon Super much more than the efficacy of Axial 50. None of these three biotypes could be controlled with 50fold of the recommended dose rate of Ralon Super. Even the ED_{90} could not be reached.

The Ile-1781-Leu in addition with Asp-2078-Gly mutations caused much higher resistance factors than the Trp-2027-Cys mutation (RF 34 compared to 88 and 144 for ED_{50}). This ranking corresponds to previous findings of DELYE et al. (2008). They concluded that all five known target site mutations in *A. myosuroides* confer resistance to fenoxaprop-p-ethyl. PETIT et al. (2010) showed for the first time that all three mutations result in resistance to pinoxaden as well. The resistance factors of Axial 50 for these mutations were much lower in our studies than for Ralon Super, especially if calculated on the basis of the ED_{50} values (RF 7-12). In general, differences became more obvious for RF values based of ED_{90} values (RF 20-168). The slope of the dose response curves for the biotypes 1 and 3 with Ile-1781-Leu and Asp-2078-Gly mutations was much flatter than for the Trp-2027-Cys mutation. The dose response curve of Axial 50 for the Trp-2027-Cys mutation looked similar to the curve for biotype 4 with EMR, like a sensitivity shift. Axial 50 was not yet applied on the location of biotype 2, but possible cross-resistance to EMR which was found for mesosulfuron-methyl and fenoxaprop-p-ethyl cannot be excluded. However the metabolism rate for fenoxaprop-p-ethyl was rather low. Our results and PETIT et al. (2010) findings also suspect that the Trp-2027-Cys mutation is the major cause for the decreased sensitivity of Axial 50.

Resistance to ALS inhibitors is not a new phenomenon in *A. myosuroides*. First observations were reported from France and UK in 1983/1984, and from Germany in 2001 (HEAP, 2011). These early resistance findings (for flupyrsulfuron), especially in Germany, were mainly caused by EMR in *A. myosuroides* populations which were preselected by PS II and ACCase inhibitors in the years before. The introduction of Atlantis WG in 2004 and Broadway in 2008 additionally increased the selection pressure in the last years. Differences in the resistance mechanism of the four biotypes investigated caused high differences in the biological activity of the herbicides. As expected, EMR resulted in much lower resistance factors (RF 3-60 based on ED_{50}) than the target site mutations (RF 101->3000 based on ED_{50}). Surprisingly the resistance factors for Broadway were always higher than for Lexus and Atlantis. Actually the effectiveness of Broadway in controlling *A. myosuroides* is judged as higher compared to Lexus. Based on the dose response results, a resistance factor of RF ~10 for Atlantis WG and Broadway resp. RF 4 for Lexus is needed to observe a decreased sensitivity with the recommended dose rates. This high biological effectiveness of Atlantis WG is the reason that the moderate resistance factor of RF 4 (based on ED_{90}) is not yet of concern for the farmer of biotype 3. The Pro-197-Thr mutation caused high but much lower resistance factors than the Trp-574-Gly mutation. The Trp-574-Gly position is essential for the binding site of most of the ALS inhibitors and therefore causes high resistance factors. An efficacy level of 50 % could not be reached with 50fold of the recommended dose rate for all three compounds. Such high resistance factors (RF >3000) were also found e.g. with RF >4100 on enzyme level by PATZOLDT et al. (2001) for a dicot weed species. Earlier studies with a Trp-574-Gly mutation in *Apera spica-venti* showed RF values of only 7 resp. 13 for mesosulfuron-methyl and iodosulfuron-methyl (BOSCH, 2007) which is unusually low. Pro-197-Thr is described as unique for sulfonylurea herbicides. Cross-resistance to others are likely, but of low level (RF <10) especially for imidazolinone herbicides (TRANEL and WRIGHT, 2002). The biotype 1 was the only biotype where Broadway showed slightly lower resistance levels than Lexus 50 DF (RF 419 compared to RF 429 based on ED_{50}). However based on the resistance level it can be concluded that the observed Pro-197-Thr mutation caused a clear cross-resistance to Broadway, which includes two compounds out of the triazolopyrimidines. It is remarkable that the herbicide and crop rotation history did not vary very much between all four locations. All farmers used 4-6 times ALS compounds for *A. myosuroides* control in cereals within the last 6-8 years. Independent of the level of the resistance factors for the individual products and biotypes, in most cases low resistance factors caused already insufficient control in agricultural practice.

4.2 Herbicide strategies for the control of selected biotypes with multiple resistance mechanisms

Herbicides are effective and convenient weed management tools and make a major contribution to agricultural productivity. Experience shows that despite the overreliance on single herbicides or herbicides of the same site of action, which likely result in resistant weed populations, they remain the preferred weed management options for farmers. The lack of new herbicides with new sites of action and the loss of valuable compounds due to regulations increases the pressure on the remaining products. Multiple resistance mechanisms in weed populations become more and more reality in practice. Single herbicide applications alone or in mixtures are not sufficient anymore. Therefore, resistance management strategies cost the farmers additional money. Grass weed populations with high infestations and multiple resistance mechanisms can not be managed mid to long-term with herbicides only. Integrated weed management options need to be established including technical production measures. Short term useful remaining tools have to be combined, like mixtures and sequence applications of products with different sites of action and with the highest efficiency potential.

In the last years, soil-acting herbicides have become the backbone for the control of heavy infestations of *A. myosuroides* populations in practice. Especially flufenacet based products had the highest and most reliable efficacy potential in our research studies and also in other studies (MOSS and HULL, 2009; MENNE et al., 2012). Moss and HULL (2009) could demonstrate that flufenacet and its mixtures had the lowest variability and highest efficacy ranking of all soil-applied herbicides tested. The herbicide strategy trial showed that all other leaf-acting herbicides could still provide a certain level of control to all biotypes tested because the seed samples consist of a population mixture. In the herbicide strategy study, Atlantis WG appeared as the most effective post-emergence applied product, especially on biotypes with metabolic resistances. However reaching the last few percentages, which are needed for a sufficient control level, are cost intensive. Therefore on locations with multiple resistances the weed management becomes more complicate and challenging for the advisors and to the farmers practice and the farmers themselves.

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