Effectiveness of flufenacet in controlling resistant black-grass (*Alopecurus myosuroides* Huds.) – comparison of glasshouse and field trial results

Wirksamkeit von Flufenacet in der Bekämpfung von resistentem Ackerfuchsschwanz (Alopecurus myosuroides Huds.) – Vergleich von Ergebnissen aus Gewächshaus- und Feldversuchen

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Summary

In the last years in practice, soil acting herbicides have become the backbone for the control of heavy infestations of grass weed populations. The known unsteady efficacy of HRAC group K1, K3 and N herbicides is controversially discussed and became linked with the development of herbicide resistance. In general, the testing of soil-acting compounds even under controlled glasshouse conditions as well as the confirmation of possible resistance is difficult and needs specific methods and a careful result interpretation. The reliability of test methods is therefore of extreme importance. The reference testing procedure such as plant density, irrigation and temperature conditions have been shown to influence the activity of the soil applied herbicides and to result in extreme differences in the efficacy levels in general, but also in differences between resistant and susceptible biotypes.

In our trials, the seed density could be ranked as the most sensitive factor. Of the tested products, Cadou SC was the most consistent herbicide followed by Boxer and Stomp Aqua. Possible reduced efficacy effects in glasshouse trials could not be verified by field trial results. The evaluation of field trials from Northern Germany did not show a significant decrease in sensitivity of black-grass (*Alopecurus myosuroides* Huds.) to flufenacet or flufenact plus diflufenican. It could be shown that seasonal variation in soil moisture, amount of rainfall, temperature conditions and application timing all influenced the efficacy level in the field the most.

Keywords: Enhanced metabolic resistance, herbicide resistance, pendimethalin, prosulfocarb, soil herbicides, test methodology

Zusammenfassung

In den letzten Jahren ist die Anwendung von Bodenherbiziden zu einem zentralen Baustein für die Bekämpfung von hohen Ungrasdichten in der landwirtschaftlichen Praxis geworden. Die schwankende Wirkungssicherheit der Bodenherbizide der HRAC Gruppen K1, K3 und N unter praktischen Feldbedingungen wird teilweise konträr diskutiert und oft mit einer möglichen Resistenzentwicklung in Zusammenhang gebracht. Die allgemeine Untersuchung der Wirksamkeit von Bodenherbiziden unter kontrollierten Gewächshausbedingungen ist schwierig und bedarf einer wirkstoffspezifischen Methodenanpassung und einer entsprechenden überlegten Ergebnisauswertung. Die Vertrauenswürdigkeit der Testmethode ist deshalb von besonderer Bedeutung. Die Vielzahl der Methodenparameter, wie unter anderem Pflanzendichte, Bewässerung und Temperaturbedingungen, beeinflussen die Wirksamkeit der Bodenherbizide und können zu hohen Wirkungsunterschieden im Allgemeinen, aber auch zu Unterschieden zwischen verschiedenen Populationen führen.

In der Überprüfung der Methodenparameter stellte sich vor allem die Pflanzendichte als einflussreicher Faktor für die Wirkungsstärke der Bodenherbizide heraus. In der Reihenfolge der getesteten Produkte war Cadou SC das wirkungsstärkste und konsistenteste Produkt, gefolgt von Boxer und Stomp Aqua. Wirkungsunterschiede in den Gewächshausuntersuchungen konnten im Allgemeinen mit den Feldergebnissen nicht bestätigt werden. Eine Auswertung von Feldversuchen aus Resistenzgebieten in Norddeutschland zeigte in den letzten Jahren keine signifikante Abnahme der Wirksamkeit von Flufenacet oder Flufenacet plus Diflufenican in der Bekämpfungsleistung von Ackerfuchsschwanz (*Alopecurus myosuroides* Huds.). Jahreszeitliche Einflussfaktoren wie Bodenfeuchtigkeit, Niederschlagsereignisse, Temperaturbedingungen und das Wachstumsstadium der Ungräser zum Zeitpunkt der Applikation zeigten einen eindeutigen Einfluss auf die Wirksamkeit der Bodenherbizide.

Stichwörter: Bodenherbizide, Herbizidresistenz, Metabolische Resistenz, Pendimethalin, Prosulfocarb, Untersuchungsmethoden

1. Introduction

Herbicide resistances in grass weeds to post-emergent applied herbicides of different HRAC groups are wide spread in intensive cereal production systems in Germany and other European countries. The limited possibilities in controlling resistant black-grass (*Alopecurus myosuroides* Huds.) with leaf-acting herbicides has resulted in a renaissance of soil applied herbicides which are being applied more and more in areas with high grass weed infestation. These herbicides provide alternative modes of action which can be important in slowing down the selection pressure of single products and sites of action. Some of these compounds already have been used for several decades but only to small extent. The extensive use of soil herbicides and their known occasional inconsistent efficacy under field conditions have not resulted in a high number of resistance reports. For *A. myosuroides,* only three reports for pendimethalin and flufenacet are recorded by HEAP (2011). Many more findings are known for group K1 (trifluralin) and N (triallate) herbicides in rigid-ryegrass (*Lolium rigidum*) and wild-oat (*Avena fatua*). Two additional reports for Italian-ryegrass (*Lolium multiflorum*) and perennial-ryegrass (*Lolium perenne*) concern possible resistance to flufenacet. In general, ryegrass species are not target grass weeds on the label of flufenacet (FOE5043, 1997); and only good effects on Italian-ryegrass are recorded with high dose rates of 480-600 g ai/ha for use in sunflower (FLUFENACET, 2000). Effects on perennial grasses cannot be expected. The spread of herbicide resistance to ACCase, ALS and PS II inhibitors in grass weeds and the enlarged number of publications have sensitized farmers and consultants to the fact that reduced effectiveness of herbicides are linked more often with possible resistances. The resistance conformation testing should be proceeded following corresponding guidelines of HEAP (2005). The testing of soil acting compounds can be problematic and needs specific methods and a careful interpretation of results (MENNE and WAGNER, 2008).

The following results describe the influence of different trial parameters on the effectiveness of soil applied herbicides under controlled glasshouse conditions. The analysis of over a decade of field trial results of flufenacet-based products aimed to demonstrate whether a decrease occurs in sensitivity of *A. myosuroides* populations in resistance areas of Northern Germany.

2. Materials and methods

2.1 Selection of seed samples and bioassay parameter

A selection of different *A. myosuroides* biotypes which were suspicious regarding a decreased sensitivity to different modes of action was identified in monitoring studies. Dose response studies with seeds of these biotypes were conducted under controlled glasshouse conditions. The procedure was as follows unless otherwise mentioned: The seed samples were cleaned, pre-germinated and sown in 8 cm pots (Fa. Jiffy) filled with a field soil (loamy silt with 18.9 % sand, 58.8 % silt, 22.3 % clay, 2.2 % organic matter, pH 7.4) with four repetitions each. The pots were placed in a glasshouse with 60 % humidity and 12 h light with minimum 2200 µE/m^{2}s (sodium high pressure lamps if necessary). Sensitive reference biotypes were used for verification. The herbicides were applied at growth stage BBCH 05-10 with a standard laboratory track sprayer (teejet nozzle XR8002, pressure 2.4 bar, water amount 300 l/ha). All products [Boxer® (prosulfocarb), Cadou SC® (flufenacet), Stomp Aqua® (pendimethalin)] were sprayed with four to seven different dose rates for dose response analysis. The evaluation was done three weeks after application by visual assessment of the damages (%) in comparison to the untreated control. For method comparison, single different factors were modified:

1. Temperature conditions – with 15 °C day/10 °C night in comparison with 22 °C day/18 °C night.
2. Irrigation conditions – from above compared to from below.
3. Seed density – 100, 500, 1000 mg seeds/pot

ED_{50} values were determined by fitting herbicide efficacies determined at different application rates to the sigmoidal dose-response model equation using Model 205 of the ID Business Solutions Ltd Xlfit version 5.1.0.0 software suite.

[Boxer® registered product of Syngenta Agro GmbH, Cadou SC® registered product of Bayer CropScience Germany GmbH, Stomp Aqua® registered product of BASF SE.]

2.2 Evaluation of field trials

The field trial database was analyzed regarding the efficacy of flufenacet, as well as flufenacet in mixture with diflufenican. Only field trial results (n = 44 for flufenacet and n = 146 for flufenacet plus diflufenican) from so called suspicious "resistance areas" in Northern Germany, Lower Saxony and Schleswig Holstein were used for further analyses. The compounds were applied at different growth stages (BBCH 00-27) in autumn. The mean, minimum, and maximum values of efficacy assessment data at the beginning of vegetation in spring were calculated for each year. The data were compared with rainfall data and average temperatures that were summarized for the month September and October in three locations from Northern Germany (Wismoor, Freiburg, Blankensee) for each year.

3. Results

3.1 Bioassay parameter and dose response studies

The tested biotypes represent population mixtures of survivors from different farmers' fields from France, Germany (Lower Saxony, Marschen) and Great Britain. The biotypes were selected regarding their high enhanced metabolism to different products of HRAC group A, B and C2. Two populations also contained a certain level of ACCase target-site mutations Ile-1781-Leu and Gly-2096-Ala. The results of the dose response studies were compared to the mean ED_{50} values of two different sensitive biotypes. The dose response results for Cadou SC, Stomp Aqua and Boxer and the different biotypes were pooled because of only slight differences between the individual resistant biotypes on the one hand and the sensitive biotypes on the other. The variation of the trial procedures significantly influenced the effectiveness of the tested products under controlled glasshouse conditions. Effectiveness data of additional studies with different growth stages of *A. myosuroides* and different soils types are not presented here. The more advanced the growth stages of *A. myosuroides* were at the time of application, the lower the effectiveness level of all products. Under these conditions, Stomp Aqua was more affected by later growth stages than Cadou SC and Boxer. The highest and most stable effectiveness could be realized at BBCH 00-10 of *A. myosuroides*. The performance of all products was more heterogeneous between repetitions on soils with higher sand content as well on soils with higher organic matter. The ED_{50} values of Boxer increased with higher temperature by factor 2-3, whereas Stomp Aqua was more active under warm conditions in these studies (Tab. 1). The irrigation from above decreased the effectiveness of Stomp and Boxer. Both compounds were influenced more than Cadou SC and the ED_{50} values increased by 40-60 %. These calculations are based on the results with the lowest plant density of 100 mg seeds.

Tab. 1 Influence of temperature and irrigation conditions on calculated ED_{50} values of tested products for all biotypes tested with the lowest plant density (100 mg seeds/pot).

Tab. 1 *Einfluss der Temperatur und der Bewässerungsbedingungen auf die ED_{50} der untersuchten Produkte für alle Biotypen bei der geringsten Pflanzendichte (100 mg Samen/Topf).*

	temperature conditions			irrigation conditions		
	warm	cool	factor	from above	from below	factor
	ED_{50w}	ED_{50c}	ED_{50w}/ED_{50c}	ED_{50a}	ED_{50b}	ED_{50a}/ED_{50b}
Cadou SC	42	36	1.2	40	44	0.9
Stomp Aqua	1849	2677	0.7	2263	1599	1.4
Boxer	1765	1115	2.2	1765	1115	1.6

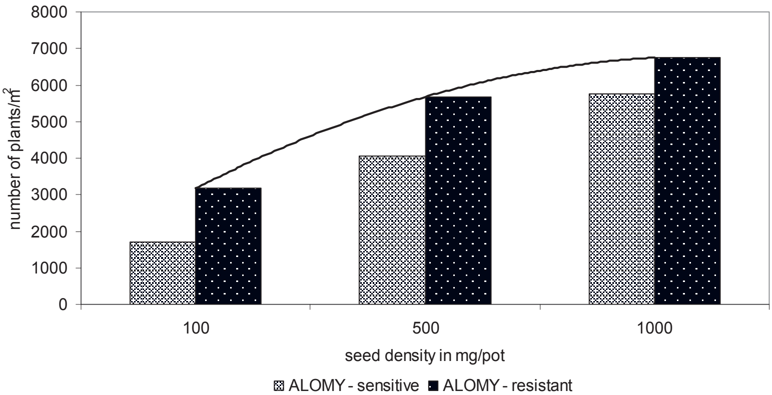

Fig. 1 Influence of seed density on the germination of *A. myosuroides* and number of plants per unit of area.

Abb. 1 *Einfluss der Aussaatdichte auf die Keimung von A. myosuroides und die Anzahl der Pflanzen je Flächeneinheit.*

The different seed densities caused different number of plants per pot. The germination rate of the sensitive biotypes was lower compared to the resistant biotypes. With the lowest seed density (100 mg seeds/pot), 8 plants/pot (1690 plants/m^2) could be realized for the sensitive biotype compared to 16 plants/pot (3172 plants/m^2) for the resistant biotypes (Fig. 1). The differences in plant densities between sensitive and resistant biotypes were smaller with higher seed densities. With 1000 mg/pot, 10 times more seeds than the lowest seed density, only 2.1 and 3.4 times in number of plants/pot of the lowest seed density could be realized. The competition reduced the germination rate and the densities were more balanced between the sensitive and resistant biotypes. The data additionally showed that the irrigation from above reduced the number of plants by 21 % within the lowest seed density. The temperature did not influence the plant density (only 3 % difference). However, even the lowest seed density already resulted in more plants than under practical field conditions. Five times more seeds per pot (which caused an increase in plants by factor 2.4 for the sensitive biotypes) resulted in 2.4 to 3.5 higher ED_{50} values for the herbicides (Tab. 2). An additional number of plants, 3.4 times higher plant density, caused an additional decrease in the effectiveness of the herbicides (factor 3.7 to 7.0 higher ED_{50} values). The compounds Stomp Aqua and Boxer were much more affected than Cadou SC in these studies.

Tab. 2 Influence of seed density and number of plants of sensitive biotypes on the effectiveness of soil applied herbicides calculated on basis of ED_{50} values and their factors (in comparison to lowest seed density; 100 mg seeds/pot).

Tab. 2 *Einfluss der Aussaatdichte und der Pflanzenanzahl der sensitiven Biotypen auf die Wirksamkeit von Bodenherbiziden auf Basis der Berechnung der ED_{50} Werte und der Faktoren (im Vergleich zur geringsten Pflanzendichte; 100 mg Samen/Topf).*

	seed density in mg per pot		
	100	500	1000
number of plants per m^2 (factor in comparison to lowest seed density)	1690	4069 (2.4)	5759 (3.4)
	ED_{50} values (factors in comparison to lowest seed density)		
Cadou SC	20	48 (2.4)	73 (3.7)
Stomp Aqua	325	772 (2.4)	1504 (4.6)
Boxer	193	679 (3.5)	1342 (7.0)

Finally, the influence of temperature, irrigation conditions and seed density resulted in inconsistent differences in the control of the resistant and sensitive biotypes. Under warm conditions, a dose response curve for Boxer on resistant biotypes and higher plant densities could not be achieved anymore so that a calculation of ED_{50} values was impossible. The irrigation from above with warm temperature compared to the irrigation from below with cool temperature nearly doubled the resistance factors (RF) for all three products (Tab. 3).

Tab. 3 Influence of irrigation conditions on the effectiveness of soil-applied herbicides to resistant and sensitive *A. myosuroides* biotypes, calculated on basis of ED_{50} values and their resistance factors (RF).

Tab. 3 *Einfluss der Bewässerungsbedingungen auf die Wirksamkeit von Bodenherbiziden auf resistente und sensitive* A. myosuroides*-Biotypen auf Basis der ED_{50}-Werte und der Resistenzfaktoren (RF).*

	cool temperature - irrigation from below			warm temperature - irrigation from above		
	ALOMY - sensitive	ALOMY - resistant	resistance factor (RF)	ALOMY - sensitive	ALOMY - resistant	resistance factor (RF)
	ED_{50s}	ED_{50r}	ED_{50r}/ED_{50s}	ED_{50s}	ED_{50r}	ED_{50r}/ED_{50s}
Cadou SC	26	50	1.9	20	78	3.9
Stomp Aqua	703	2762	3.9	466	3232	6.9
Boxer	136	1753	12.9	247	4572	18.5

3.2 Analysis of field trial data

The analyses of the field trial database was narrowed down to the so called suspicious "resistance areas" in Northern Germany, Lower Saxony and Schleswig Holstein. All trials were taken into account where flufenacet and flufenacet plus diflufenican were applied in autumn at growth stages BBCH 00-27. The summary results in Figure 2 illustrate the high variance in the effectiveness of flufenacet within and among years. Only a limited number of results (n = 44) were available for flufenacet alone.

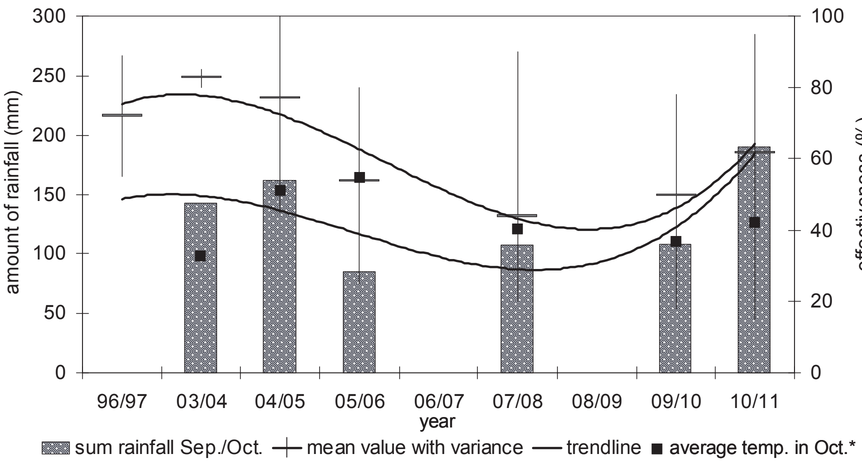

Fig. 2 Effectiveness of flufenact on *A. myosuroides* under field conditions dependent on rainfall and temperature.

Abb. 2 *Wirksamkeit von Flufenacet auf A. myosuroides in Abhängigkeit von Niederschlag und Temperatur unter Feldbedingungen.*

(* average temperature as trend, without scale in figure)

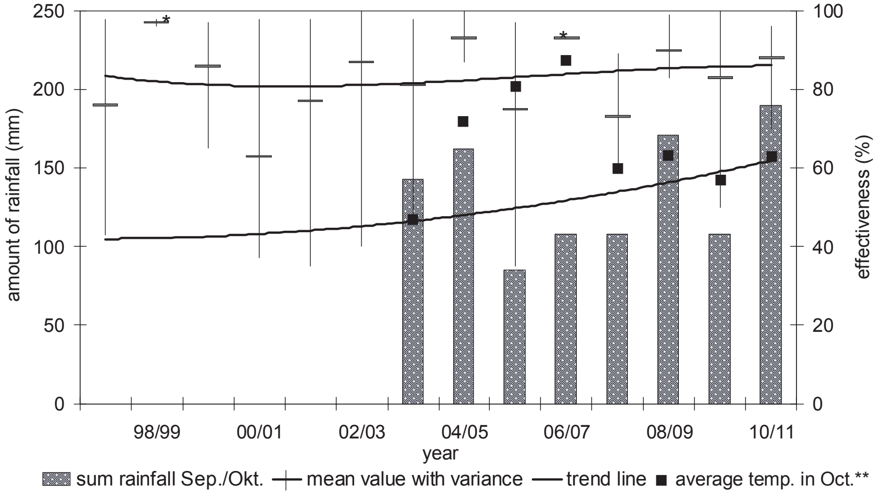

Fig. 3 Effectiveness of flufenacet plus diflufenican on *A. myosuroides* under field conditions dependent on rainfall and temperature.

Abb. 3 *Wirksamkeit von Flufenacet plus Diflufenican auf A. myosuroides in Abhängigkeit von Niederschlag und Temperatur unter Feldbedingungen.*

(* only reduced number of data; **average temperature as trend, without scale in figure).

It becomes obvious that the effectiveness of flufenacet was strongly correlated with the amount of rainfall before and after the application in September/October in each year. The trend lines for the effectiveness and the rainfall describe the same curve shape. The variation in each year also depended on the growth stage of *A. myosuroides* at the time of application. The temperature conditions were a further factor. At lower temperatures, the efficacy of flufenacet was higher than under warmer conditions. The influence interacts with the soil moisture conditions. A comparable trend could be observed for flufenacet plus diflufenican too (Fig. 3). The database of field trial results was much broader for flufenacet plus diflufenican (n = 146) than for flufenacet alone. Only for the years 1999 and 2007, the number of data points was too low (n = 1-2). In general, the variation of effectiveness of flufenacet plus diflufenican was much lower in comparison to flufenacet. The trend line was flatter and the variation in each year depended on the growth stage of *A. myosuroides* at the time of application and on the rainfall conditions. The data for the amount of rainfall were available from 2003 onwards only. Years with low effectiveness levels with ≤ 80 % and high variation (e.g. 2006, 2008, 2010) were hallmarked by low rain fall levels ~100 mm only. Much higher rainfall with > 150 mm in the years 2005, 2009 and 2011 caused higher control levels of flufenacet plus diflufenican.

4. Discussion

4.1 Bioassay parameter and dose response studies

The evolution and spread of herbicide resistance has resulted in the development of different methodologies based on specific key questions, modes of action and/or specific compounds. Investigations of effectiveness of herbicides under controlled glasshouse conditions are conducted today by a range of institutions and quite often result in controversial discussions. Ring tests between different institutions, which were carried out in the past, resulted partly in controversial data, mainly caused by technical equipment, light and climate conditions (PETERSEN et al., 2010). Most of these effectiveness tests of herbicides are carried out post-emergent in advanced growth stages of plants. Effectiveness tests with soil-applied herbicides are much more critical and cannot be directly compared with tests of leaf-acting herbicides. In these tests, the methodological parameters and physical-chemical properties of compounds are essential for the validity of results.

In general, soil-acting compounds like flufenacet, pendimathelin and prosulfocarb have low water solubility and higher Koc values which influence their soil behavior decisively. Dry soil conditions and irrigation from below keep the compounds close to the soil surface. This effect is even stronger in soils with high clay and organic matter content. In contrast, the application on wet soils and irrigation from above result in leaching of compounds in deeper soil layers. Sandy soils and low organic matter contents encourage this process (MENNE and WAGNER, 2008). Soil acting herbicides require soil moisture and cooler conditions for their optimal effectiveness. Under warm conditions the plants grow quickly before the products have a chance to be effective.

The highest influence on the effectiveness of the compounds could be observed with the seed density of *A. myosuroides*. Higher seed densities cause a higher number of plants which have to share the compound amount with each other. The low amount of compound per plant, the plant competitive interaction, such as faster and deeper root growth and etiolating of plants, cause an insufficient effectiveness. This phenomenon could be observed for leaf-acting herbicides as well. The observation of lower germination rate of sensitive biotypes compared to resistant biotypes is not unusual. From experience, it is assumed that sensitive biotypes gradually lose their germination ability with time. The effect of plant density was most obvious for Boxer which caused an increased ED_{50} level by a factor of 7. A high number of plants do not help in getting a meaningful dose response for any herbicide tested. The most meaningful trial results for all three products were achieved with the lowest seed density, under cool temperature conditions and irrigation from below. The resistant biotypes were so insufficiently controlled by Stomp Aqua and Boxer such that cross-resistance to these high metabolic resistance *A. myosuroides* biotypes could be confirmed with RF of 4 und 13. Cadou SC was still efficient under these conditions. However, higher differences for all compounds were observed under unfavorable trial conditions, such that a possible sensitivity shift on biotypes with high metabolic resistance cannot be excluded. MOSS and HULL (2009) and KLINGENHAGEN (2010) could demonstrate that flufenacet and its mixtures had the lowest variability and highest efficacy ranking of all soil-applied herbicides tested, followed by Boxer and Stomp. For tests of soil acting compounds the following factors should be considered among others:

1. Moderate soil types with clay contents < 30 %, sand contents < 40 %, 2-3 % of organic matter to avoid strong adsorption, but also to avoid leaching with high sand contents.
2. It is recommended to choose plant densities which do not exceed 1000 plants/m^2 inclusive known sensitive and resistant standard biotypes.
3. Irrigation from below to avoid leaching conditions, but activation of herbicides 24 hours after application with 5-10 mm of water from above.
4. Avoid application of herbicides on absolutely dry soils which result in fast and strong adsorption.
5. Cool conditions with maximum of 15-18 °C during day and 8-10 °C night.

Each factor should not be separately seen on its own. The interaction and balancing of all mentioned factors contribute to meaningful trial results.

4.2 Field trial data

The effectiveness of soil acting herbicides is strongly dependent on the soil and climate conditions at the time of application. The single products are not applied alone because of their limited activity on grasses and overall weed spectrum. The application of soil herbicides in corn in spring result in side effects to *A. myosuroides* only, when soil moisture is missing and under warmer temperatures (KLINGENHAGEN, 2011). All three products are rated by WOLBER (2010) with side effects to good effectiveness at their recommended dose rates in cereals in autumn. A very good efficacy rating is not expected based on their dependence on soil moisture conditions, temperature and plant growth stage. However soil-acting herbicides have become the backbone for the control of heavy infestations of *A. myosuroides* populations in practice. Flufenacet plus diflufenican and especially flufenacet alone showed a high variation in their effectiveness in field trials. However a sensitivity shift of *A. myosuroides* to flufenacet-based products in the northern regions of Germany, where resistance to ACCase, ALS and PSII inhibitors is wide spread, could not be confirmed yet on field levels. The variation in first instance depended on soil moisture conditions, temperature and plant growth stage at the time of application. Other factors will have an additional influence like herbicide dose, persistence, spraying accuracy, seedbed conditions, weed emergence patterns, crop competition etc. (MOSS and HULL, 2009). The HRAC K1, K3 and N products and their mixtures are still quite effective and reduce the plant density of the first flush of germination of *A. myosuroides* plants.

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