Comparison of different black-grass populations (*Alopecurus myosuroides* Huds.) in their susceptibility to herbicides under field conditions

Vergleich verschiedener Ackerfuchsschwanz-Populationen (Alopecurus myosuroides Huds.) in ihrer Empfindlichkeit gegenüber Herbiziden unter Freilandbedingungen

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Summary

In autumn 2009 and 2010, black-grass samples from different regions in Germany were sown into a field near Münster. The sandy soil of the field was free of black-grass seeds until the sowing date. The aim of the trial was to compare the fitness and resistance value of different black-grass populations under outdoor conditions. Particularly the impact of pre-emergence herbicides was of interest. Different efficiency factors of CADOU SC® (flufenacet) against the tested black-grass populations were observed. The disparities in the level of control were even greater under the use of STOMP AQUA® (pendimethalin), BOXER® (prosulfocarb) and ARELON TOP® (isoproturon). A hundred percent control of all black-grass populations in both seasons was only achieved by the use of KERB FLO® (propyzamide). It was visible that the black-grass populations that were difficult to control had a powerful constitution, even better than that of those populations that turned out to be highly susceptible to the herbicide treatments. The fall results in both seasons of the experiment (2009/2010 and 2010/2011) were consistent. This was also confirmed for the efficacy results for foliar herbicides in spring. Subject to field history, there was a wide range in sensitivity to the tested herbicides. Black-grass populations from coastal regions were poorly controlled by FOCUS ULTRA® (cycloxydim) or SELECT 240 EC® (clethodim) + PARA SOMMER® (paraffin oil), but were highly susceptible to MOTIVELL® (nicosulfuron). Contrary results were observed with reference to the black-grass populations from the inland. These populations were derived from fields where the crop rotation contains of 66 % cereals and 33 % maize. The populations from the coast were sampled in crop rotations dominated by cereals or cereals followed by oil seed rape. Poor efficacy of ATLANTIS OD® (mesosulfuron + iodosulfuron) was observed in black-grass populations from the coastal regions as well as from the inland.

Key words: Crop rotation, fitness, flufenacet, mesosulfuron, propyzamide, prosulfocarb, quizalofop-p

Zusammenfassung

Im Herbst der Jahre 2009 und 2010 wurden Samen unterschiedlicher Ackerfuchsschwanz-Populationen auf einem Feld in der Nähe von Münster ausgesät. Bis zur Saat war der Boden, ein Sandboden, frei von Ackerfuchsschwanz-Samen. Ziel des Versuches war es, die ökologische Fitness und den Resistenzgrad unterschiedlicher Ackerfuchsschwanz-Populationen unter Freilandbedingungen miteinander zu vergleichen. Hierbei ging es besonders um die Wirksamkeit von Bodenherbiziden. Es wurde deutlich, dass die Wirkung von CADOU SC® (Flufenacet) gegen die geprüften Ackerfuchsschwanz-Populationen unterschiedlich war. Deutlich größer waren die Unterschiede im Bekämpfungserfolg bei Einsatz von STOMP AQUA® (Pendimethalin), BOXER® (Prosulfocarb) und ARELON TOP® (Isoproturon). Eine hundertprozentige Bekämpfung aller Ackerfuchsschwanz-Herkünfte konnte in beiden Versuchsjahren nur durch den Einsatz von KERB FLO® (Propyzamid) erreicht werden. Es war zu beobachten, dass die schwer bekämpfbaren Ackerfuchsschwanz-Herkünfte über eine gute Konstitution verfügten. Diese war besser als die Konstitution der Herkünfte, die sehr empfindlich auf die Herbizidbehandlungen reagierten. Die Ergebnisse aus den Herbstbehandlungen der Versuchsjahre 2009/2010 und 2010/2011 waren konsistent. Dies gilt auch für die Bekämpfungserfolge, die nach den Behandlungen im Frühjahr erhoben wurden. In Abhängigkeit von der Schlaghistorie war die Empfindlichkeit der unterschiedlichen Ackerfuchsschwanz-Herkünfte sehr unterschiedlich. Ackerfuchsschwanz-Populationen aus den Küstenregionen wurden mit FOCUS ULTRA® (Cycloxydim) oder SELECT 240 EC® (Clethodim) + PARA SOMMER® (Paraffinöl) schlecht bekämpft, waren aber empfindlich gegenüber MOTIVELL® (Nicosulfuron). Gegenteilig war die Reaktion der Ackerfuchsschwanz-Populationen aus dem Binnenland. Diese Populationen entwickelten sich in Fruchtfolgen, die zu 66 % aus Getreide und zu 33 % aus Mais bestehen. Die Populationen der Küstenregionen sind durch Getreide- bzw. Getreide-Raps-Fruchtfolgen geprägt. Eine unzureichende Wirkung von ATLANTIS OD® (Mesosulfuron + Iodosulfuron) konnte sowohl bei den Populationen aus den Küstenregionen wie aus dem Binnenland beobachtet werden.

Stichwörter: Cycloxydim, Fitness, Flufenacet, Freiland, Fruchtfolge, Mesosulfuron, Propyzamid

1. Introduction

The control of black-grass (*Alopecurus myosuroides* Huds.) in cereals is a major issue in North-West Europe (DROBNY et al., 2006). Especially on heavy soils, control problems have arisen during the last 30 years. Prior to the availability of crop-selective grass-herbicides like TRIBUNIL® (methabenzthiazuron), ARELON® (isoproturon) or DICURAN® (chlortoluron), these fields with heavy soils were mainly used as grassland (MENCK, 1968). Since then, further products for black-grass control came onto the market. With the frequent use of these compounds, resistant black-grass populations were selected (RUBIN, 1996). This becomes most obvious in production systems which provide high amounts of black-grass seeds. Early drilling, insufficient control and minimum tillage are adequate tools to boost a black-grass population. A high initial frequency of resistance alleles in a population, outbreeding, dominance of inheritance, a short persistence of seed bank in the soil and the lack of a fitness penalty for resistant versus susceptible biotypes of a weed species are further factors that favor the occurrence and increase of herbicide resistance (ZWERGER and WALTER, 1994). So far, it has been proven that black-grass populations have developed resistance and cross-resistance to a number of herbicides belonging to different modes of action. This is true for photosynthesis II (PS II) inhibitors such as isoproturon and chlortoluron. These herbicides are classified by the Herbicide Resistance Action Committee (HRAC) in group C2. Another widespread resistance is that to acetyl-CoA carboxylase (ACCase) inhibitors like fenoxaprop-P-ethyl, clodinafop and pinoxaden (HRAC group A). Furthermore, the number of black-grass populations resistant to acetolactate-synthase (ALS) inhibitors like flupyrsulfuron and mesosulfuron is increasing (MOSS and HULL, 2009). Most of the populations analyzed so far have developed metabolic resistance mechanisms (DROBNY et al., 2006). These populations are able to metabolize different herbicides from different chemical classes and with different modes of action (MENDES and DEPRADO, 1997). To prevent a rapid development of resistance, it is a general advice to use pre-emergence herbicides, presuming that the risk of selecting plants that are resistant to this mode of action is low. Among the pre-emergence herbicides, flufenacet is the most important component to control black-grass in winter cereals. Flufenacet is one of the active components in HEROLD SC®, MALIBU® (flufenacet + pendimethalin) and BACARA Forte® (flufenacet + diflufenican + flurtamone). That is why the results of glasshouse trials, where a significantly reduced efficacy of CADOU SC® against five black-grass biotypes was found, were alarming. A 90 % reduction of the shoot biomass was achieved with application rates between 0.79 and 4.45 l/ha CADOU SC®. Calculated resistance factors based on the EC_{50} values are reported between 8.8 and 49.5 (KRATO and PETERSEN, 2010). These resistance cases are reported under www.weedscience.org (PETERSEN, 2010). These scientific findings and a discussion with colleagues (who have to deal with the most challenging black-grass populations) were the background of the trial program.

2. Materials and methods

The field trials were carried out in 2009/2010 and 2010/2011. The black-grass seeds that where drilled in autumn 2009 were collected in summer 2009. For the trial in 2009/2010, we received samples of populations from six different locations in Germany. One from the east coast ("Dingebauer"), another from the west coast of Schleswig-Holstein ("Schleich-Saidfar") and two samples from Münsterland ("Coesfeld" and "Warendorf"). Moreover, seeds were obtained from Rhineland ("Bonn") and a sensitive standard population from the seed supplier Appel ("Appel"). The black-grass populations from Schleswig-Holstein and Münsterland had been taken from so-called "problematic sites". In addition to that, for the trial in 2010/2011, we received black-grass samples from two locations near Hannover ("Seemann" and "Haarstrich"), from Ostfriesland ("Pewsum"), from the Schwäbische Alp ("Heisrath") and from a field near Unna in Westphalia ("Grünewald"). "Dingebauer" was drilled twice (harvest 2009 and 2010). The population "Schleich-Saidfar" from 2010 was collected from a different field from that in 2009. The black-grass seeds were mixed with winter wheat (100 seeds per m²). Sowing was conducted September 29th in 2009 and October 4th in 2010. The field was located in Münster, Germany. The soil is a sandy soil with 1.9 % humus and a pH of 5.8. The soil contents of P_2O_5 (25 mg/100 g soil), K_2O (18 mg/100 g soil), and Mg (5 mg/100 g soil) are in a balanced proportion. The field is owned by the Chamber of Agriculture and did not contain any black-grass seeds until the day of drilling. The different black-grass populations were sown in parallel strips of 40 m length and a width of 1.2 m. To obtain different depths of deposition, each strip was drilled twice at depths of 0.5 to 2.5 cm. According to a germination test, around 1000 fertile black-grass seeds per m^2 were drilled. The herbicide applications were conducted crosswise to the strips. Each herbicide was sprayed over a width of 2 m. The pre-emergence applications were carried out October 14^{th} in 2009 at growth stage BBCH 00-10 and October 6^{th} in 2010 at growth stage BBCH 00. In both seasons, there were satisfactory soil and weather conditions for the herbicidal activity of the pre-emergence herbicides. The spraying was done with trial spraying device, equipped with an airmix antidrift 11003 nozzle. The herbicides were sprayed in 300 liters water per ha with a pressure of 2.3 bar. On the spraying dates mentioned, Cadou SC®, Herold SC®, Stomp Aqua®, Boxer® and Arelon Top® were applied. In autumn 2010, Cadou SC® was replaced by Fuego® (metazachlor). Kerb flo® was sprayed November 18^{th} of 2009 at growth stage BBCH 12-13 and November 22^{nd} in 2010 at growth stage BBCH 11-13. The spring applications were carried out April 27^{th} in 2010 at growth stage BBCH 25-29 and March 8^{th} in 2011 at growth stage BBCH 13-25. Atlantis OD®, Roundup Ultra Max® (glyphosate), Targa Super® (quizalofop-p), Focus Ultra®, Select 240 EC®, + Para Sommer® (paraffin oil) and Motivell® (nicosulfuron) were applied as spring applications. In addition to that a tankmixture of Laudis® (tembotrione) + Gardo Gold® (s-metolachlor + terbuthylazine) + B 235® (bromoxynil) was applied April 21^{st} in 2011 at growth stage BBCH 25-29. The impact of the herbicides was estimated over the growing period; the herbicidal efficacy was asessed as % visual biomass reduction. The final assessment was done June 16^{th} in 2010 and on June 1^{st} in 2011, respectively.

Tab. 1 Commercial name, active ingredient(s) and potency of the used products.

Tab. 1 *Handelsname, Wirkstoff(e) und Wirkstoffgehalte der eingesetzten Produkte.*

Product	Active ingredient	Potency (g/l or kg)
Arelon Top	isoproturon	500
Atlantis OD	mesosulfuron + iodosulfuron	10 + 2
B 235	bromoxynil	235
Boxer	prosulfocarb	800
Cadou SC	flufenacet	500
Focus Ultra	cycloxidim	100
Gardo Gold	s-metolachlor + terbuthylazin	313 + 188
Herold SC	flufenacet + diflufenican	400 + 200
Kerb Flo	propyzamid	400
Laudis	tembotrione	44
Motivell	nicosulfuron	40
Select 240 EC	clethodim	242
Stomp Aqua	pendimethalin	455
Targa Super	quizalofop-p	50

3. Results

After drilling on September 29^{th} 2009, the first black-grass sprouts emerged on 16^{th} of October. At the time of emergence, the visual appearance of different populations was quite uniform. Four days later, differences became more obvious. Especially "Dingebauer" was impressive. Plants appeared to be faster, thicker and greener than those of the other populations. Compared to "Dingebauer", the susceptible standard "Appel" made a weak impression. In the following spring season, the conditions for germination were adverse. One week after drilling, it was getting cold and wet and a frost period started and lasted until the beginning of January 2010. At the end of January, the first plants had reached the growth stage 10 and on February 6^{th}, the drilling rows were clearly visible and at least

100 plants per m² of each population had emerged. Spring-like weather until the 17th of February provided good growing conditions until frost down to minus 8 °C occurred. At the beginning of March 2010, a large number of black-grass plants were lost by winter-kill. Particularly the plant densities of populations "Appel", "Bonn" and "Grünewald" which could be assumed to be susceptible to the herbicides tested, were significantly reduced. On the other hand, the populations "Warendorf", "Schleich-Saidfar", "Dingebauer" 2009/2010 and "Pewsum", which responded less susceptible to herbicides, were not as strongly affected and "Seemann" overcame the frost period in an unaffected way. The populations "Haarstrich" and "Heisrath" do not fit into this scheme.

As "Seemann" also showed no response after being treated with ATLANTIS OD® or MOTIVELL®, plants were tested by Dr. Jean Wagner via molecular genetic analysis. He found Leu574-resistance in all (twenty) samples examined. In fourteen of the twenty samples, the Leu574-resistance was found on both alleles (homozygous).

The efficacy of herbicide applications against the different black-grass populations is shown in the tables below.

Tab. 2 Efficacy of the herbicide applications in autumn 2009 against different black-grass (*Alopecurus myosuroides* Huds.) populations.

Tab. 2 *Wirkung der Herbizidbehandlungen im Herbst 2009 gegen unterschiedliche Herkünfte von Ackerfuchsschwanz (Alopecurus myosuroides Huds.).*

	Herbicide	**CADOU SC**	**HEROLD SC**	**STOMP AQUA**	**BOXER**	**ARELON TOP**	**KERB FLO**
	Rate (l/ha)	**0.5**	**0.6**	**4.0**	**4.0**	**3.0**	**1.5**
Population	**Ears/m²**	**% efficacy (biomass reduction)**					
"Appel"	300	100	100	80	80	70	100
"Warendorf"	430	90	95	0	0	0	100
"Schleich-Saidfar"	380	90	97	10	0	70	100
"Dingebauer"	430	80	85	10	0	50	100
"Coesfeld"	380	90	97	10	0	40	100
"Bonn"	400	100	100	60	70	60	100

Tab. 3 Efficacy of herbicide applications in spring 2010 against different black-grass populations (*Alopecurus myosuroides* Huds.).

Tab. 3 *Wirkung der Herbizidbehandlungen im Frühjahr 2010 gegen unterschiedliche Herkünfte von Ackerfuchsschwanz (Alopecurus myosuroides Huds.).*

	Herbicide	**ATLANTIS OD**	**ROUNDUP ULTRA MAX**	**TARGA SUPER**	**FOCUS ULTRA**	**SELECT 240 EC**	**MOTIVELL**
	Rate (l/ha)	**1.2**	**2.0**	**1.0**	**2.5**	**0.5 + 1.0**	**1.0**
Population	**Ears/m²**	**% efficacy (biomass reduction)**					
"Appel"	300	98	100	100	100	100	100
"Warendorf"	430	40	100	98	100	100	95
"Schleich-Saidfar"	380	80	100	65	75	85	98
"Dingebauer"	430	85	100	15	15	70	95
"Coesfeld"	380	75	100	95	99	100	100
"Bonn"	400	98	100	100	100	100	100

Tab. 4 Efficacy of herbicide applications in autumn 2010 against different black-grass populations (*Alopecurus myosuroides* Huds.).

Tab. 4 *Wirkung der Herbizidbehandlungen im Herbst 2010 gegen unterschiedliche Herkünfte von Ackerfuchsschwanz (Alopecurus myosuroides Huds.).*

	Herbicide	**HEROLD SC**	**FUEGO**	**STOMP AQUA**	**BOXER**	**IPU**	**KERB FLO**
	Rate (l/ha)	**0.6**	**1.5**	**4.0**	**4.0**	**3.0**	**1.5**
Population	**Ears/m²**	**% efficacy (biomass reduction)**					
"Appel"	12	100	100	100	90	97	100
"Warendorf"	450	70	65	30	0	0	100
"Schleich-Saidfar"	320	90	70	70	65	35	100
"Dingebauer 09"	540	65	35	30	25	65	100
"Dingebauer 10"	630	75	40	25	0	50	100
"Bonn"	75	100	100	95	100	70	100
"Pewsum"	450	70	55	35	20	80	100
"Haarstrich"	90	95	75	70	70	n.a.	100
"Seemann"	1000	85	85	45	40	40	100
"Heisrath"	80	75	60	50	40	45	100
"Grünewald"	47	100	60	55	55	70	100

Tab. 5 Efficacy of the herbicide applications in spring 2011 against different black-grass populations (*Alopecurus myosuroides* Huds.).

Tab. 5 *Wirkung der Herbizidbehandlungen im Frühjahr 2011 gegen unterschiedliche Herkünfte von Ackerfuchsschwanz (Alopecurus myosuroides Huds.).*

	Herbi-cide	**LAUDIS + GARDO GOLD + B 235**	**ATLAN-TIS OD**	**ROUND-UP ULTRA MAX**	**TARGA SUPER**	**FOCUS ULTRA**	**SELECT 240 EC**	**MOTI-VELL**
	Rate (l/ha)	**2.0 + 3.0 + 0.3**	**1.2**	**2.0**	**1.0**	**2.5**	**0.5 + 1.0**	**1.0**
Population	**Ears/m²**	**% efficacy (biomass reduction)**						
"Appel"	12	100	100	98	100	100	100	100
"Warendorf"	450	35	55	90	70	100	100	100
"Schleich-Saidfar"	320	40	85	90	0	35	10	100
"Dingebauer 09"	540	40	97	95	30	65	30	100
"Dingebauer 10"	630	50	93	85	15	65	20	100
"Bonn"	75	98	100	98	70	100	100	100
"Pewsum"	450	35	45	70	0	65	15	100
"Haarstrich"	90	80	65	80	20	100	90	75
"Seemann"	1000	80	0	65	70	100	99	0
"Heisrath"	80	85	75	65	0	30	0	20
"Grünewald"	47	96	100	97	70	90	90	100

4. Discussion

Even though the application conditions for pre-emergence herbicides were optimal, satisfactory control of those populations originating from so-called problematic sites ("Warendorf", "Schleich-Saidfar", "Dingebauer 09/10", "Coesfeld", "Pewsum", "Haarstrich", "Seemann" and "Heisrath") could not be achieved. Only the "easy-to-control" populations ("Appel", "Bonn" and "Grünewald") were completely controlled by the use of CADOU SC® or HEROLD SC®. This was observable in 2009/2010 as well as in 2010/2011. The lack of herbicidal efficacy was not as high as in the glasshouse trials mentioned by KRATO and PETERSEN (2010), but it became obvious that the different resistance factors are of great importance for black-grass control in the field. Regarding the use of STOMP AQUA®, the differences in control level between the populations were enormous. The aforementioned populations were poorly controlled. Nearly the same result was found after the application of BOXER®. This was unexpected because according to the HRAC-classification, BOXER® is outstanding in class N and should be dissimilar to the K1 and K3 herbicides CADOU SC® and STOMP AQUA®. Nevertheless, the results agree with the findings of a three-year-lasting outdoor pot-trial (MOSS and HULL, 2009). Another congruent result is that populations which are not sufficiently controlled by CADOU SC® and HEROLD SC® are (more or less) resist to ATLANTIS OD®. According to MOSS and HULL (2009), the subsidiary influence of diflufenican for black-grass control could be observed. The distinction between "resistant" and "sensitive" black-grass populations was not that easy after application of ARELON TOP® (isoproturon). The control of populations from the coast was relatively successful. Possibly this can be related to the limited use of isoproturon on these sites in the past. The results of the KERB FLO® treatment were really encouraging: Total control in both seasons against all populations. Even during the die-off period, no differences were visible. This fact is in line with studies in which no difference in efficacy after use of different propyzamide rates between "Peldon" (black-grass population known to be resistant against chlortoluron and fenoxaprop) and "LARS" (black-grass population known to be susceptible to chlortoluron and fenoxaprop) could be measured (EDMONDS and CASELEY, 1997).

In case of ATLANTIS OD®, one could see a scheme similar to flufenacet. All populations from the "problematic sites" were inadequately controlled. The unequal level of control between the two seasons was probably a result of the disparate circumstances at spraying time. One difference between these "hard-to-control" populations becomes evident when considering the great differences in control between FOCUS ULTRA® and MOTIVELL®. The populations from the midland were still susceptible to FOCUS ULTRA® with indication for increasing resistance against MOTIVELL®. Contrary to that was the plant development of the populations from the coastal regions. It can be assumed that this was caused by differences in crop rotations and corresponding spraying regimes. The obvious disparities in efficacy between FOCUS ULTRA® and SELECT 240 EC® + PARA SOMMER® were, from the author's point of view, due to the weather conditions: SELECT 240 EC® + PARA SOMMER® could benefit from the warm climate in spring 2010. In the following season, it was quite cold during the period of application. Under these conditions, superior control was achieved with FOCUS ULTRA®. The weather constellation may also explain the poor impact of ROUNDUP ULTRA MAX® in 2011. Apart from this, the populations turned out to be unequally susceptible. This should not lead to a resistance discussion about black-grass and glyphosate but it may be an indication that, under unfavorable conditions, there is a narrow margin between success and failure of a glyphosate application. Studies using 52 black-grass biotypes lead to the conclusion that there was a range in the ED_{50} factor between 0.09 and 2.65 (KRATO and PETERSEN, 2010)

The 2011 spring application of LAUDIS® + GARDO GOLD® + B 235® should have been carried out at earlier growth stages. Nevertheless, the black-grass populations responded differently to this treatment even though none of the populations had been in contact with LAUDIS® before.

It is right and necessary to alternate between herbicides with different mode of action. But the impact of this alternation is limited. To stretch the duration of usefulness of herbicidal active ingredients, the product of number of seeds per field x number of herbicide treatments should be as low as possible. "Resistance is a number game" (KAUDUN, 2011).

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