Impact of non-target-site-resistance on herbicidal activity of imazamox on black-grass (*Alopecurus myosuroides* Huds.) in comparison to other ALS-graminicides

Einfluss der Nicht-Wirkortresistenz auf die herbizide Wirkung von Imazamox auf Ackerfuchsschwanz (Alopecurus myosuroides Huds.) im Vergleich zu anderen ALS-Graminiziden

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Abstract

A black-grass (*Alopecurus myosuroides* Huds.) resistance-monitoring conducted by BASF in 2010 - 2012 revealed a high number of accessions with resistance against imazamox. However, application of imazamox-based products in a winter crop was limited to winter beans in France and United Kingdom only until the introduction of the Clearfield®-production system in autumn 2012 in winter oilseed rape. It is therefore assumed that the resistance mechanisms were probably selected by the frequent use of ACCase- and ALS-inhibitors in winter crop rotations during the last 2 decades. Resistance level for each product-biotype combination was calculated according the "R"-classification system (S, R?, RR, RRR) by directly comparing the product performance on a biotype versus untreated control. Majority of resistant biotypes did not show a target-site mutation at the known codon Pro197 or Trp574. In order to better evaluate the impact of Non-Target-Site-Resistance (NTSR) on the activity of BEYOND (imazamox), ATLANTIS WG (mesosulfuron+iodosulfuron) and ABAK (pyroxsulam), biotypes who have shown an ALS-target-site mutation were removed from further analysis.

At the dose rate of 35 g ai/ha BEYOND provided good activity on susceptible biotypes of black-grass almost matching up with ATLANTIS WG and ABAK. However, activity of BEYOND declined stronger on biotypes classified as R? or RR for that product, while ATLANTIS WG and ABAK hardly showed any decline in control on this group of biotypes when applied at the recommended dose rate. It is assumed that the underlying NTSR-mechanism is not effective enough yet to confer resistance to ATLANTIS WG and ABAK, but on BEYOND. In contrast, biotypes classified as R? for ATLANTIS WG did show a stronger impact on the activity of BEYOND and ABAK then of ATLANTIS WG. These differences in control level probably do translate into differences in selection pressure as well.

Keywords: Mesosulfuron, NTSR, pyroxsulam, resistance

Zusammenfassung

In einem Ackerfuchsschwanz-Monitoring, das von der BASF in 2010-2012 durchgeführt wurde, zeigte eine hohe Anzahl an Herkünften eine Resistenz gegenüber Imazamox. Anwendungen von imazamox-basierten Produkten in Winterungen waren bis zu diesem Zeitpunkt jedoch auf Winterbohnen in Frankreich und Großbritannien beschränkt. Erst mit der Einführung des Clearfield®-Produktionssystems im Herbst 2012 in Winterraps ist eine Anwendung in einer flächenmäßig bedeutsamen Winterung möglich. Es ist daher anzunehmen, dass die in diesen Herkünften vorliegenden Resistenz-Mechanismen durch die häufige Anwendung von ACCase- und ALS-Hemmern in Winterungen während der letzten beiden Jahrzehnte selektiert wurden. Die Einstufung des Resistenzgrades der geprüften Produkte auf die einzelnen Biotypen wurde dabei nach dem „R"-Klassensystem (S, R?, RR, RRR) vorgenommen. Die Mehrheit der resistenten Biotypen zeigte keine Wirkort-Resistenz an den bekannten ALS-Mutationsstellen Pro197 und Trp574. Um den Einfluss der Nicht-Wirkort-Resistenz auf die Aktivität der Produkte BEYOND (imazamox), ATLANTIS WG (mesosulfuron+iodosulfuron) und ABAK (pyroxsulam) besser beurteilen zu können, wurden die entsprechenden Biotypen mit ALS-Wirkort-Resistenz nicht in die weitere Analyse einbezogen.

Bei der Aufwandmenge von 35 g ai/ha erzielte BEYOND eine gute Wirkung auf sensitive Ackerfuchsschwanz-Biotypen, wobei annähernd das Wirkungsniveau von ATLANTIS WG und ABAK erzielt werden konnte. Allerdings fiel die Wirkung von BEYOND deutlich ab, wenn Behandlungen auf die für dieses Produkt zugeordneten Biotypen der Resistenzklassen R? oder RR erfolgten. Die Wirkung von ATLANTIS WG und ABAK auf diese Biotypen wurde bei Anwendung der einfachen Aufwandmenge hingegen kaum beeinflusst. Es kann angenommen werden, dass die vorliegenden Nicht-Wirkort-Resistenzmechanismen zu wenig ausgeprägt waren um eine Resistenz gegenüber ATLANTIS WG und ABAK herbeizuführen. Im Gegensatz dazu fiel die Wirkung von BEYOND und ABAK im Vergleich zu ATLANTIS WG stärker ab, wenn die Biotypen gegenüber ATLANTIS WG die Einstufung R? zeigten. Es ist davon auszugehen, dass die beobachteten Unterschiede im Wirkungsgrad sich auch als Unterschiede im Selektionsdruck wiederfinden werden.

Stichwörter: Mesosulfuron, NTSR, Pyroxsulam, Resistenz

Introduction

Herbicide resistance in black-grass is already an established threat in winter annual cropping systems in the maritime area of Western Europe, where resistance has constantly been evolved over the last decades. Besides the mode of action specific target-site resistance, other mechanism, described and cumulated as Non-Target-Site Mechanism (NTSR) are gaining importance because of their unpredictable resistance to herbicides with different mode of action (PETIT *et al.*, 2010). Black-grass population resistant to ACCase-inhibitors very often do possess high frequent target-site mutation side by side with non-target-site mechanism. In ALS-resistant population occurrence and frequency of the known mutation at Pro197 and Trp574 is lower, revealing a strong impact of NTSR-mechanism (SIEVERNICH *et al.*, 2013).

It was demonstrated that NTSR is under polygenetic control, but accumulation of up to at least three NTSR loci in a single plant could be necessary to confer resistance. This leads to the assumption that NTSR loci are accumulating within individual plants depending on the selection pressure, which results in a gradual increase in the frequency of resistant plants and in the average resistance level of an individual plant to a given herbicide (DÉLYE *et al.*, 2010).

The introduction of the Clearfield®-production system in winter oilseed rape enables farmers for the first-time to control a broad spectrum of problematic dicotyledonous weeds, including brassicaceae by the use of products based on the ALS-inhibitor imazamox. The additional activity on sensitive population of black-grass (*Alopecurus myosuroides* Huds.) raises the question whether this use in winter oilseed rape generates additional selection pressure on-top of the commonly utilized ALS-graminicides in cereals.

Material and methods

Seeds of black-grass were sampled during 2010-2012 throughout Europe to investigate the resistance pattern against various graminicides with different modes of action. Sampling was done in a non-randomized manner with major emphasis on Germany, France, United Kingdom, Belgium and Netherland, including locations with product failure, suspicious resistance cases and trial sites.

Cultivation of black-grass was done primarily by direct sowing into plastic or Jiffy-pots (Jiffy Products International B.V., NL) of about 8cm in diameter. At low germination rates, transplanting of pre-cultivated plants were done at about 1-2 leaf stage approximately 4-5 days before application to establish a minimum of 8 plants per pot.

Greenhouse trial design includes 3 replications per treatment and the inclusion of a sensitive reference which was uniform through the years at all test locations.

Application was done at the 2-3 leaf stage of black-grass using 0.5x, 1x and 2x of the maximum recommended dose rate of various post-emergence ALS-graminicides of different chemical classes (Tab. 1). All products were applied with their recommended adjuvant.

Final efficacy evaluation was done 3-4 weeks after application as a visual assessment in % plant damage in a treated pot compared to the untreated control.

Tab.1 Geprüfte Produkte und deren Aufwandmengen.

Tab.1 *Tested products and their dose rates.*

Product	**Active Substance(s)**	**Chemical Class**	**Dose rate**	**Product/ha**	**g ai/ha**	**adjuvant**
BEYOND	Imazamox	Imidazolinone	0.5x	145 ml/ha	17,5	DASH EC
			1x	290 ml/ha	35	
			2x	580 ml/ha	70	
ATLANTIS WG	Mesosulfuron + Iodosulfuron	Sulfonylurea	0.5x	250 g/ha	7,5 + 1,5	MERO
			1x	500 g/ha	15 + 3	
			2x	1000 g/ha	30 + 6	
ABAK	Pyroxsulam	Triazolopyrimidine	0.5x	125 g/ha	9,38	RME
			1x	250 g/ha	18,75	
			2x	500 g/ha	37,5	

Product efficacy on a biotype was compared to the same treatments on the sensitive standard in the trial and ascribed to one of four resistance classes (S, R?, RR, RRR) according to MOSS (1999).

SNP (Single Nucleotide Polymorphism) analysis of biotypes classified as R?, RR or RRR was initiated to investigate on any potential target-site mutation at position Pro197 and Trp574 of the ALS-enzyme.

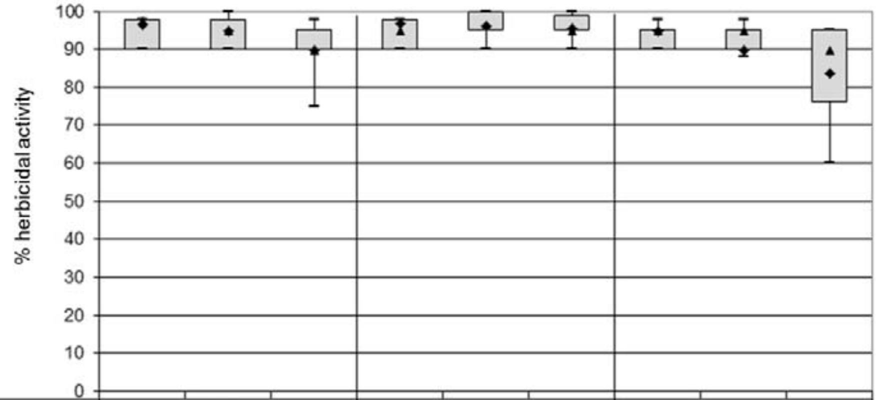
With the aim of this analysis, to focus on the impact of NTSR on the activity of various ALS-inhibitors, biotypes in which an ALS-target-site resistance was detected have been excluded. However, the set of biotypes still includes those biotypes with an ACCase target-site resistance.

Product performance was analyzed by comparing the activity of the tested products against a defined group of black-grass biotypes. Definition of the group of biotypes was done according to a selected resistance class of a product (e.g. all R?-classified biotypes of BEYOND).

The qualitative parameters (1) product and (2) dose rate as well as their interaction were tested for significant differences with Tukey's HSD (Honestly Significant Difference) test at $p = 0.05$, performing a two-way ANOVA with the statistic software *R* (R Development Core Team 2010) and the *R* add-on package *agricolae* (DE MENDIBURU, 2013)

Results

A high uniformity in the performance of the tested products on the sensitive reference assures a high trial and data quality. The “R”-system requested to have a minimum activity on the sensitive reference of 80%. Herbicidal activity, as presented in Figure 1, of the three ALS-inhibitors ATLANTIS WG, ABAK and BEYOND on the sensitive black-grass reference was at the expected high level with a control of ≥ 90% at the 1x dose rate. At the reduced dose rate of 0.5x herbicidal activity declined slightly and revealed some differentiation between the products with a lower stability and reliability in the activity of BEYOND compared to the other products (Fig. 1).



product	Atlantis + Mero			Capri + adjuvant			Beyond + Dash EC		
kg,l fp/ha	1	0,5	0,25	0,5	0,25	0,125	0,58	0,29	0,145
g ai/ha	30+6	15+3	7,5+1,5	37,5	18,75	9,4	70	35	17,5
Mean ◆	97	95	90	97	96	96	95	90	84
Median ▲	98	95	90	95	97	95	95	95	90

Abb. 1 Herbizide Wirkung von ALS-Inhibitoren auf Ackerfuchsschwanz (sensitiver Standard).

***Fig. 1** Herbicidal activity of ALS-inhibitors on black-grass (sensitive standard).*

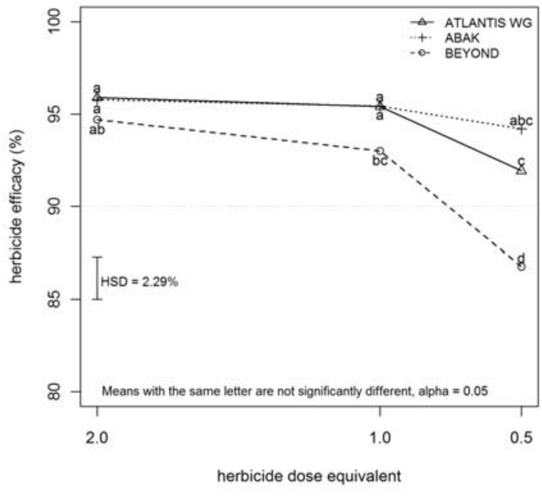


Abb. 2 Herbizide Wirkung von ALS-Inhibitoren auf Ackerfuchsschwanz (sensitive Einstufung).

***Fig. 2** Herbicidal activity of ALS-inhibitors on black-grass (sensitive classification).*

By selecting biotypes sampled at different locations in Europe (excluding the sensitive reference) and classified as being susceptible to BEYOND (n=60) a similar result was obtained as with the sensitive reference. At the 2x and 1x dose rate activity level of all products was quite similar (95-96% resp. 93-96%), while at the 0.5x dose rate activity started to differentiate more (87-96%). At the 1x rate, but even more pronounced at the 0.5x dose rate, black-grass control of BEYOND was significantly lower than of ATLANTIS WG and ABAK (Fig. 2).

In a further approach, black-grass biotypes were selected who did show an R?-classification for the use of BEYOND (n = 21). The R?-class is defined to show an early indication that resistance may be developing (MOSS *et al.*, 1999).

BEYOND revealed a clear and significant dose response with black-grass activity stretching from 62% at 0.5x, 83% at 1x and 90% at the 2x dose rate. In contrast, herbicidal activity of ATLANTIS WG and ABAK was still at a high level with 89-92% at the 1x dose rate, but with a certain decline in control at the 0.5x rate. The loss in performance at the 0.5x rate was already significant for ATLANTIS WG but not for ABAK (Fig. 3).

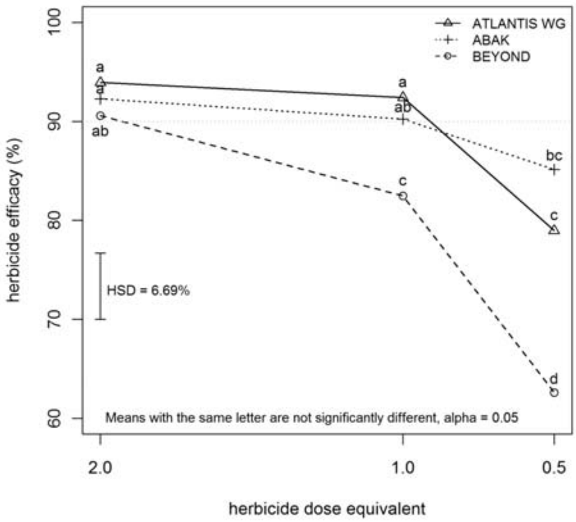


Abb. 3 Herbizide Wirkung von ALS-Inhibitoren auf Ackerfuchsschwanz (R?-Einstufung).

***Fig. 3** Herbicidal activity of ALS-inhibitors on black-grass (R?- classification).*

On black-grass biotypes with an RR-classification on BEYOND (n = 50), activity level strongly drops further to 43% at 0.5x, 62% at 1x and 79% at the 2x dose rate. However, declining activity at all dose rates of ATLANTIS WG and ABAK was observed as well with 71-72%, 79-85% and 85-92% control for dose rates of 0.5x, 1x and 2x respectively (Fig. 4).

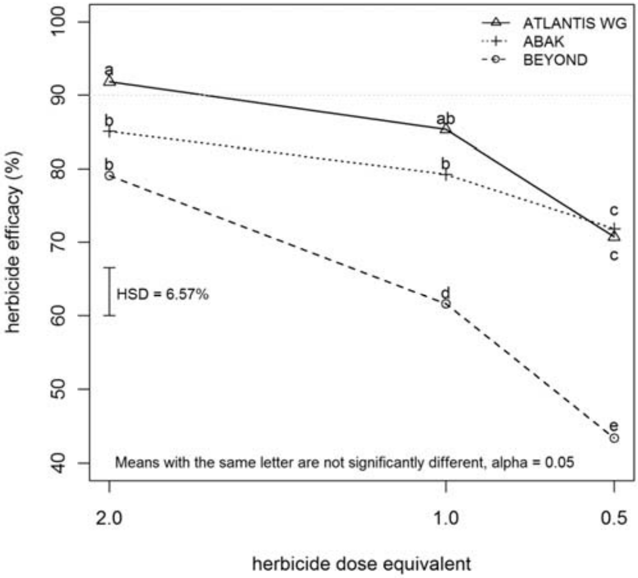


Abb. 4 Herbizide Wirkung von ALS-Inhibitoren auf Ackerfuchsschwanz (RR-Einstufung).

Fig. 4 *Herbicidal activity of ALS-inhibitors on black-grass (RR- classification).*

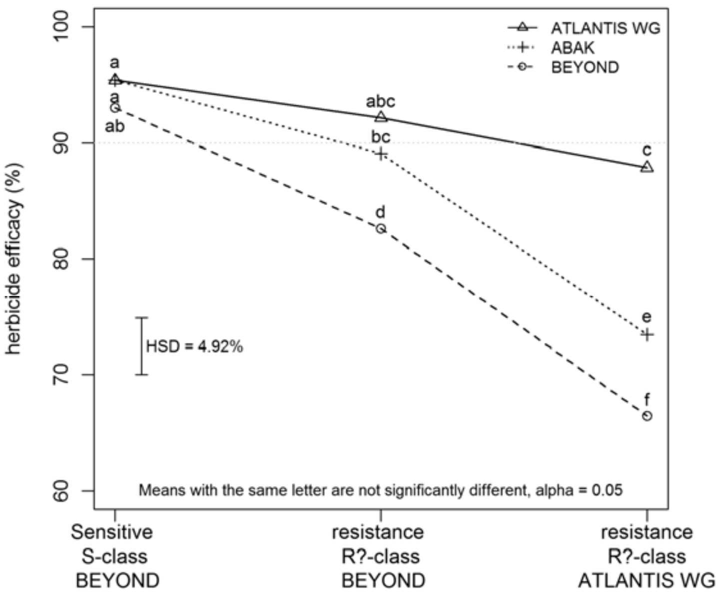


Abb. 5 Herbizide Wirkung von ALS-Inhibitoren auf Ackerfuchsschwanz unter Berücksichtigung verschiedener Stufen der Resistenzausprägung bei Vorliegen einer Nicht-Wirkortresistenz.

Fig. 5 *Herbicidal activity of ALS-inhibitors on black-grass considering different levels of resistance classes in presence of non-target-site resistance.*

Figure 5 shows a summary of Figure 3 and 4 at the recommended field rate of the respective tested herbicides.

Comparing biotype groups of black-grass classified as either R? on BEYOND (n = 21) or ATLANTIS WG (n = 16), a much stronger decline in activity was observed for the R?-group of ATLANTIS WG than for those of BEYOND. Activity performance dropped for BEYOND 93%/83%/66%, ABAK 95%/89%/73% and ATLANTIS WG 95%/92%/88% for the S- and R?-class of BEYOND and R?-class of ATLANTIS WG respectively (Fig. 5).

Discussion

Imazamox based products were hardly used in European winter crop rotation before the introduction of CLEARFIELD®-winter oilseed rape in 2012. The only exception is the use of a ready-mixture with pendimethalin in winter beans in some areas of France and United Kingdom. Thus, the number of black-grass population not possessing an ALS-target-site mutation, but already conferring resistance to the active substance imazamox is surprisingly high. However, this is in line with the assumption that NTSR revealing an unpredictable risk for herbicide performance independent on the mode and site of action and the chemical class of the active substance. NTSR mechanism conferring to the more or less expressed resistance to imazamox in black-grass must have been selected by other grass weeds herbicides, such as ACCase- and other ALS-inhibitors frequently used in winter crop rotations.

The inherent activity of an active substance on a weed is influenced by several factors, such as rate of uptake and translocation, stability within the plant and inhibition level of the target enzyme(s). Active substances providing similar control on a weed may vary in one or several of these characteristics, which could then discriminate in the response of evolving NTSR-mechanism. Resistance profile of a population depends on the NTSR loci conferring resistance, their accumulation in the plant, inheritability and spread within the population (PETIT, 2010). As such, different populations or even individual plants within a population may vary in their response to various herbicides, requesting the testing of a larger number of accessions to provide a better overview how these principle findings apply under field conditions. A comparative setup of resistance monitoring and a more stringent use of the generated data do already provide additional useful information.

Activity of BEYOND declined stronger at the different resistance classes (R? & RR on BEYOND) compared to ATLANTIS WG and ABAK. One can assume that the underlying NTSR-mechanism are not effective enough yet to confer resistance to ATLANTIS WG and ABAK, but on BEYOND. A further accumulation of NTSR loci in individual plants of the population seems to be necessary for further evolving resistance towards these herbicides. This might happen already in those biotypes classified as R? for ATLANTIS WG. However, activity of BEYOND and ABAK were even stronger affected by the NTSR-mechanism present in these biotypes.

Resistance evolution is selection in progress and it requires selective pressure for a further enrichment or (re-) combination of NTSR resistance genes within the population. It is yet not understood whether and how different selection pressure due to the use of single products, tank-mixtures or sequence application do have an influence on the dynamic of accumulation, combination and spread of NTSR in a population.

The data presented does provide information on the different response of BEYOND, ATLANTIS WG and ABAK on NTSR present in a broad collection of black-grass accessions from different locations throughout major winter cropping areas in Western Europe. It is assumed that the difference in control level does translate into a difference in selection pressure as well.

In Germany, imazamox is registered in CLEARFIELD®-winter oilseed rape with a maximum dose rate of 12.5 g ai/ha, which is even well below the 0.5x dose rate (17,5 g ai/ha) tested. Thus, the declining activity response to the different groups of black-grass biotypes is even stronger, with a corresponding reduction in selection pressure.

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