Sektion 3: Herbizidresistenz - Management

Section 3: Herbicide resistance - Management

Impact of imazamox containing herbicides on the development of resistance in black-grass (*Alopecurus myosuroides* Huds.)

Einfluss von Imazamox-haltigen-Herbiziden auf die Resistenzentwicklung bei Acker-Fuchsschwanz (Alopecurus myosuroides Huds.) in einer Raps-Getreidefruchtfolge

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Abstract

Winter oilseed-rape was the most common crop in Western Europe where no ALS-inhibitor was used. Due to the introduction of Clearfield winter oilseed-rape varieties the use of ALS-inhibitors also in oilseed-rape is possible. If the broader use of ALS-inhibitors increases the selection pressure on herbicide resistant weeds and increases their occurrence in the crop rotation is the question of this investigation. Therefore, an outdoor container trial (á 350 l, 0.7 m^2) was performed starting in autumn 2011. A typical crop rotation of winter wheat/oilseed-rape/winter wheat was simulated in the following three years. Three different black-grass biotypes with characterised resistance pattern and 5 different herbicide programs were analysed. The black-grass biotypes showed different target-site resistance against ACCase- and/or ALS-inhibitor, as well as metabolic resistance. Before and after each treatment the numbers of black-grass plants per container were counted. Also the numbers of heads were counted before harvest. Additionally genetic analysis due to PCRs and pyrosequencing of ten survivors per container and year were performed.

Till now results of the winter wheat and oilseed-rape cultivation were obtained. Herbicide efficacy was between 77 and 98% for the treatments during the winter wheat cultivation. The genetic analysis showed nearly similar portion of TSR in the black-grass populations when compared with the initial frequencies. Only one container showed no TSR.

The comparison of the herbicide programs sprayed during the oilseed-rape cultivation showed the best results for all black-grass biotypes for the application of: Metazachlor + dimethenamid (BBCH 09/10), imazamox + quinmerac + Dash (BBCH 14) and propyzamide (BBCH 21/22).

Keywords: ACCase-inhibitor, ALS-inhibitor, Clearfield oilseed-rape, target-site resistance (TSR), herbicide rotation

Zusammenfassung

Winterraps war bislang die bedeutendste Ackerbaukultur in Westeuropa in der kein ALS-Inhibitor eingesetzt werden konnte. Mit der Einführung von Clearfield Winterrapssorten ändert sich dies mit der Verwendungsmöglichkeit von Imazamox-haltigen Herbiziden. Eine wichtige Frage in diesem Zusammenhang ist, ob durch den erweiterten ALS-Herbizideinsatz in einer Fruchtfolge der Selektionsdruck steigt und in der Folge mit vermehrtem Auftreten herbizidresistenter Unkräuter zu rechnen ist. Um dieser Frage nachzugehen, wurde im Herbst 2011 ein Gefäßversuch (a 350 l, 0,7 m^2) unter Freilandbedingungen angelegt. Simuliert wurde eine typische dreigliedrige Fruchtfolge (Winterweizen/Raps/Winterweizen). Es wurden drei verschiedene Ackerfuchsschwanz-Biotypen mit charakterisierten Resistenzen und 5 verschiedene Herbizidvarianten in den einzelnen Kulturen über die Fruchtfolge untersucht. Die Biotypen unterschieden sich bezüglich ihrer Zielortresistenz gegenüber ACCase und/oder ALS Inhibitoren und zusätzlicher metabolischer Aktivität. Vor und nach jeder Applikation wurde die Anzahl lebender Ackerfuchsschwanzpflanzen in den Gefäßen bestimmt. Blattproben von zehn überlebenden Pflanzen je Kübel dienten der genetischen Analyse der Zielortresistenz. Die Analyse von einzelnen Pflanzen ermöglicht Aussagen über die Frequenz und den Heterozygotiegrad der Zielortresistenz. Der Abgleich von Ausgangsfrequenz mit den TSR-Funden nach jeder Kultur ermöglicht die

Entwicklung des TSR-Anteils in den Populationen abhängig von den Herbizidvarianten darzustellen und lässt somit Rückschlüsse auf eine mögliche Selektion resistenter Biotypen zu. Neben den überlebenden Pflanzen wurde auch die Anzahl der Ackerfuchsschwanzähren kurz vor der Ernte bestimmt. Es liegen Daten der Winterweizen- und der Winterrapskultur vor. Diese zeigen für den Herbizideinsatz im Winterweizen Wirkungsgrade von 77 bis 98 %. Die Blattproben ergaben vergleichbare Anteile TSR in den Populationen mit den Ausgangswerten. Jedoch fanden sich auch bei ursprünglich nicht ALS-TSR Populationen bis zu 8 % Zielortresistenzen an der Mutationsstelle 197. Nur in einem Gefäß wurde keine TSR festgestellt. Die Erntemengen variierten zwischen den Biotypen und lagen zwischen durchschnittlich 7,3 und 9,3 t/ha, wobei eine starke Korrelation zwischen Erntemenge und Wirkungsgrad (r=0,7372) feststellbar war. Ein Vergleich der Herbizidvarianten im Winterraps zeigt die höchste Wirksamkeit bei allen ALOMY Biotypen für die Variante bei der die Wirkstoffe Metazachlor + Dimethenamid (BBCH 09/10), Imazamox + Quinmerac + Dash (BBCH 14) und Propyzamid (BBCH 21/22) in den jeweiligen ALOMY Stadien appliziert wurden.

Stichwörter: ACCase Hemmer, ALS Hemmer, Clearfield Raps, Zielortresistenz (TSR), Herbizidwechsel

Introduction

The imidazolinone herbicide imazamox belongs to the ALS inhibitor class (HRAC B). The herbicide is registered in Europe in different crops (Clearfield and some legume crops) up to 75 g ai/ha. Imazamox possesses the main activity via leaf uptake, while soil activity is of minor importance. The solo active ingredient will not be registered in oilseed-rape (OSR). The main formulation partner is metazachlor (+/- quinmerac). Both herbicides are synergistic in their efficacy on many important weeds in OSR. For example *Capsella bursa-pastoris*, *Lepidium draba*, *Bunias orientalis*, *Thlaspi arvense* and *Barbarea vulgaris* are well controlled. Clearfield herbicides will also control emerged volunteer cereals, thus the treated area for volunteer cereal control with Dim/Fop herbicide will decrease and subsequently also reduce the selection pressure with this herbicide class (Group A). As a consequence, this herbicide class would be then available for the in-crop use in cereals/sugar beet without changing the overall selection pressure in the crop rotation (PRESTON, 2003). The objective of this study is to clarify the impact on resistance development of Clearfield herbicides in a typical OSR crop rotation (winter wheat [WW] / OSR/ WW). Therefore, outdoor container trial with known black-grass populations was performed in Bingen. Genetic analysis of target-site frequency in black-grass is provided via BASF. The outdoor container experiment is designed for three years and started in October 2011.

Material and Methods

<u>Outdoor container trial</u>

The outdoor container trial comprises 30 containers (0.7 m^2), three black-grass biotypes, two replications and two different herbicide treatments in the first year (WW) and five different herbicide treatments in the second year (OSR). The three biotypes (“102”, “148” and “201”) are supplied by BASF and differ according their phenotypic- and genetic resistance patterns (Tab. 1).

Tab. 1 Characterization of ALOMY biotypes used in the outdoor container trial.

***Tab. 1** Charakterisierung der ALOMY Herkünfte aus dem Gefäßversuch.*

			resistance mechanism							
			ACCase-TSR					**ALS-TSR**		**NTSR**
year	No.	origin	1781	2027	2041	2078	2096	197	574	
2010	102	GB	88%	0%	25%	5%	3%	11%	0%	x
2010	148	DE	0%	13%	0%	0%	13%	0%	0%	x
2010	201	FR	96%	0%	0%	0%	0%	0%	0%	x

To establish a seed stock in the containers, 8.0 g black-grass seeds consist of 7.2 g susceptible (Herbiseeds) and 0.8 g resistant black-grass seeds (following a ratio of 9:1) were mixed in the soil in about 5 to 10 cm depth. The same amount of black-grass seeds in the same mixture were sown in rows alternating with winter wheat (250 seeds per m^2). Figure 1 schematically shows the 30 containers with the different biotypes and herbicide treatments.

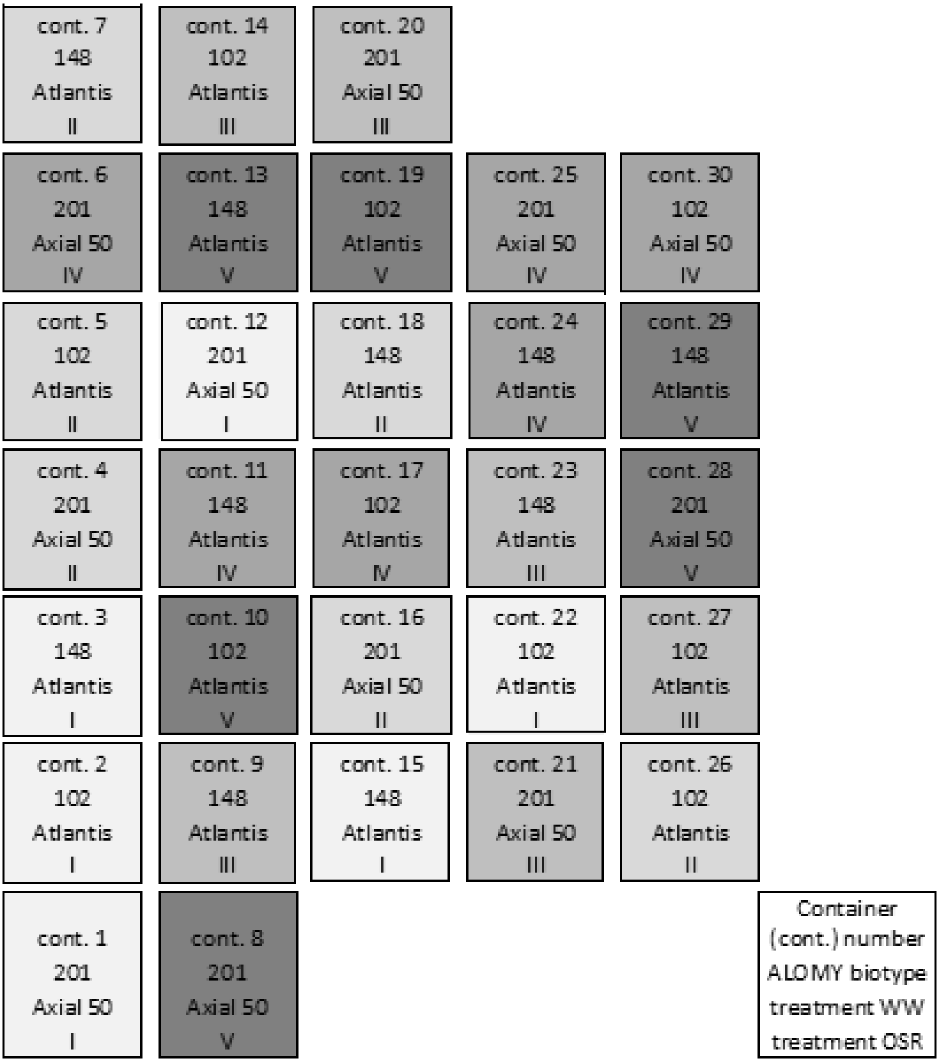

***Fig. 1** Experimental set-up for the herbicide treatments in autumn and winter 2012-2013.*

Abb. 1 Aufbau des Gefäßversuchs für die Herbizidbehandlungen im Herbst und Winter 2012-2013

The herbicides used as well as the dosages and the growth stage of ALOMY at the application date are given in Table 2. Parameters of application are given in Table 3.

Tab. 2 Herbicide treatments for the container trial.

Tab. 2 *Herbizidbehandlungen des Gefäßversuchs.*

	ACC-TSR (BT 201)	**ALS-TSR (BT 102)**	**EMR (BT 148)**
WW	Axial 50 (1.0 l/ha)	Atlantis WG (400 g/ha) + Dash	Atlantis WG (400 g/ha)+ Dash
OSR	**I II III IV V**	**I II III IV V**	**I II III IV V**
WW	Atlantis WG (500 g/ha)	Axial 50 (1.2 l/ha)	Axial 50 (1.2 l/ha)

Number OSR	**Treatment product**	**dose**	**active ingredient**	**BBCH ALOMY**
I	Butisan Gold	2.5 l/ha	metazachlor + dimethenamid + quinmerac	09/10
	Focus Ultra	1.0 l/ha	cycloxydim	14
	Dash	1.0 l/ha		
II	Butisan Kombi	2.5 l/ha	metazachlor + dimethenamid	09/10
	BAS 831 00H	1.0 l/ha	imazamox + quinmerac	14
	Dash	1.0 l/ha		
III	Butisan Kombi	2.5 l/ha	metazachlor + dimethenamid	09/10
	BAS 831 00H	1.0 l/ha	imazamox + quinmerac	14
	Dash	1.0 l/ha		
	Kerb flo	1.875 l/ha	propyzamid	21/22
IV	BAS 798 01 H*	2.0 l/ha	metazachlor + imazamox + quinmerac	12
	Focus Ultra	1.0 l/ha	cycloxydim	14
	Dash	1.0 l/ha		
V	BAS 798 01H*	2.0 l/ha	metazachlor + imazamox + quinmerac	12
	Dash	1.0 l/ha		
	Kerb flo	1.875 l/ha	propyzamid	21/22

Tab. 3 Parameters of the herbicide applications.

Tab. 3 Parameter der Herbizidapplikationen.

Nozzle	Air mix 110-025 Flat Fan
Water volume	200 l ha^{-1}
Pressure	2.1 bar
Speed	4.5 km h^{-1}

The numbers of emerged black-grass plants per container were counted on February 29th 2012. To reduce the risk of completely kill the population in the first year only 80% of the recommended field dose of the two herbicides were used. Those were 80% of the recommended field dose of the ALS-inhibitor Atlantis WG (mesosulfuron + iodosulfuron), equate 400 g ha-1 for the biotypes 102 and 148 and 80% of the recommended field dose of the ACCase-inhibitor Axial 50 (pinoxaden), and equate 1.0 l ha-1 for the biotype 201. The containers were treated with a one-wheel plot sprayer on March 12th 2012 in BBCH 24 (Tab. 2) according to the different herbicide treatments.

Numbers of survived black-grass plants per container were counted four weeks after herbicide application on April 16th 2012. Before flowering, the containers were isolated by pollen proved sleeves to avoid cross-pollinating between the biotypes. Irrigation was done as required by a drip irrigation hose. The numbers of heads of every single container were counted ten weeks after the application on May 21th 2012. In the containers, black-grass seeds were allowed to mature and fall on the ground. The winter wheat was harvested after nearly all black-grass seeds were fallen on July 16th 2012.

<u>Oilseed-rape 2013:</u>

Tillage was done by hand after the harvest of the winter wheat. A glyphosate treatment was applied on August 13th 2012 against dicots and volunteer cereals, before sowing of the oilseed-rape. Due to seed dormancy, black-grass rarely germinated and it was therefore not necessary to count ALOMY plants before the stubble treatment of glyphosate. The sowing of Clearfield oilseed-rape was done on the August 27th 2012. Fifty germinable seeds/m² (germination rate = 90%) were sown in five rows. The Clearfield oilseed-rape variety “Pioneer X08W9821” was used. Irrigation started on September 3rd and was applied if necessary. Five different herbicide treatments (resp. sequences) were conducted with two repetitions per black-grass biotype (Tab. 2).

The pre-emergence herbicides Butisan Gold and Butisan Kombi were applied on September 4th 2012. No black-grass plants emerged before this treatment. On September 28th 2012 Focus Ultra and the herbicides “BAS 798 01H” and “BAS 831 00H” were applied to the corresponding containers. Kerb flo was sprayed on December 6th 2012. The numbers of black-grass plants per container were counted four times. They were counted three days before the treatment of the post-emergence herbicides on September 25th and three weeks later on October 18th. In spring 2013 the surviving black-grass plants were counted on March 18th and April 4th. The numbers of heads per container were counted on June 5th 2013. Oilseed-rape was harvested on July 27th.

<u>Genetic analysis</u>

For genetic analysis leaf samples of ten different survived black-grass plants per container were taken during the winter wheat cultivation (on 16th and 17th April 2012) and during the oilseed-rape cultivation (April 23rd 2013) and separately dried at room temperature. DNA-extraction and genetically analysis were provided by BASF. PCRs and Pyrosequencing were performed for the ACCase mutation site 1781 and the ALS mutation site 197.

Results

<u>Outdoor container trial</u>

The number of emerged black-grass plants in winter wheat range between 843 (biotype 148) and 232 plants (biotype 102) (Tab. 4). Biotype 102 showed in general lowest germinating rate. All in all, the mean values of black-grass plants per containers were quite consistent between the three biotypes. After the application of Axial 50 biotype 201 showed the highest surviving rate with 12%. The both biotypes treated with Atlantis WG, 102 and 148 showed less survivors, especially biotype 102 with a mean value of 19 black-grass plants per container. This is the same for the number of heads. However, there were survivors in every container, producing seeds for the following years.

Tab. 4 Number of germinated and survived ALOMY plants as well as number of heads/container in winter wheat 2011/12 according to the biotypes.

Tab. 4 *Anzahl der aufgelaufenen und überlebenden ALOMY Pflanzen, sowie die Ährenanzahl pro Gefäß im Winterweizen (2011/12) für die jeweiligen Herkünfte.*

	biotype	**herbicide**	**mean value**	**min**	**max**
number of emerged ALOMY	102	ALS	475	232	593
	148	ALS	541	394	843
	201	ACCase	528	395	712
number of survivors	102	ALS	19 (4%)*	11	28
	148	ALS	33 (6%)*	17	54
	201	ACCase	62 (12%)*	35	107
number of heads	102	ALS	82	16	263
	148	ALS	138	70	249
	201	ACCase	663	354	994

* Percentage of survivors from the number of emerged black-grass plants

Results of the herbicide treatments at the last counting date (April 22nd 2012) in the OSR cultivation are given in Figure 2. The last herbicide treatment was done on December 6th 2012. A further reduction of black-grass plants for all treatments and biotypes was observed compared to the autumn counting. Herbicide efficacy varied in-between the treatments and among the biotypes. The post-emergence treatment in T1 (Focus Ultra) showed high efficacy for all biotypes. Numbers of black-grass plants per container varied between 24.5 (biotype 102) and 169.5 (biotype 201). Treatment 2 (BAS 831 00H) showed less black-grass plants per container for all biotypes expect biotype 102 (31.5 plants) when compared to treatment 1. Treatment 3 showed the best black-grass control for all biotypes when compared to the other herbicide treatments. For all biotypes less than 10 plants per container were found. There were a high number of black-grass plants left after the pre-emergence treatment with Butisan Kombi when compared to treatment 1 and 2. After the treatment with BAS 831 00H + Dash the numbers of black-grass plants clearly decreased. In Treatment 4 no pre-emergence but two post-emergence herbicides were applied. BAS 798 01H and Focus Ultra were applied at the same time. The efficacy of the tank-mixture was worse to all other treatments. 120 and 280 plants per container survived (biotype 102 and 201 respectably). Treatment 5 was the second treatment without pre-emergence herbicides. When compared to the results of treatment 4, where Focus Ultra was additionally applied, the solo application of BAS 798 01H was nearly as effective as the combined application.

The numbers of heads per pot, counted on June 6th 2013 are given in Figure 3. Treatment 3 obtained a complete black-grass control where no black-grass biotype developed one single head. Also treatment 5 resulted in no black-grass heads for biotype 102 and 148. Biotype 201 developed only five heads. The highest number of heads was found for biotype 102 and 201 in treatment 4 where no sufficient black-grass control was obtained. Also treatment 1 and 2 did not control the different black-grass biotypes sufficiently. Depending on the resistance status of the biotypes, treatment 1 showed better results for the weak ACCase-TSR biotype 148 when compared to the strong ACCase-TSR biotype 201. For treatment 2 it was the opposite situation.

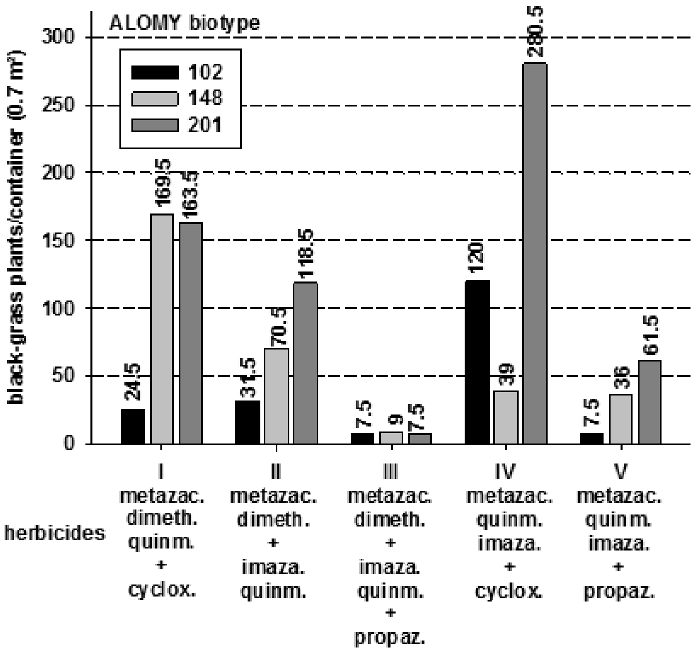

Fig. 2 *ALOMY plants in CL-OSR depending on biotype and herbicide programme (22.04.2013).*

Abb. 2 ALOMY Pflanzenanzahl im CL-Raps nach Herkunft und Herbizidapplikationsfolge (22.04.2013).

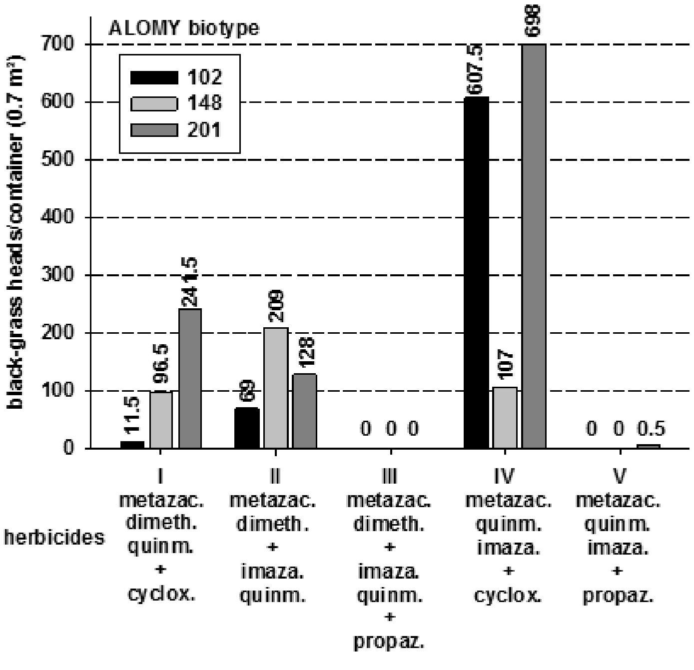

Fig. 3 *ALOMY heads/container in CL-OSR depending on biotype and herbicide programme.*

Abb. 3 ALOMY Ähren pro Container im CL-Raps nach Herkunft und Herbizidapplikationsfolge.

Genetic analysis

After the application in winter wheat 2012 a decrease of approximately 66% of TSR-ALOMY plants was found for biotype 102 (treated with Atlantis) at the ACCase mutation site 1781. Also for biotype 201 (treated with Axial) a reduction of TSR (1781)-ALOMY plants of 13% was detected. Biotype 148 (treated with Atlantis) did not show any TSR mutation at the position 1781 before our trials. According our analysis, we found in 7% of the tested plants in 2012 TSR (1781). The amount of ALS-TSR (197) was for all biotypes lower than 10%. Figure 4 shows the number of ALOMY plants with ACCase 1781 mutation found in spring 2013 in OSR, depending on biotype and herbicide.

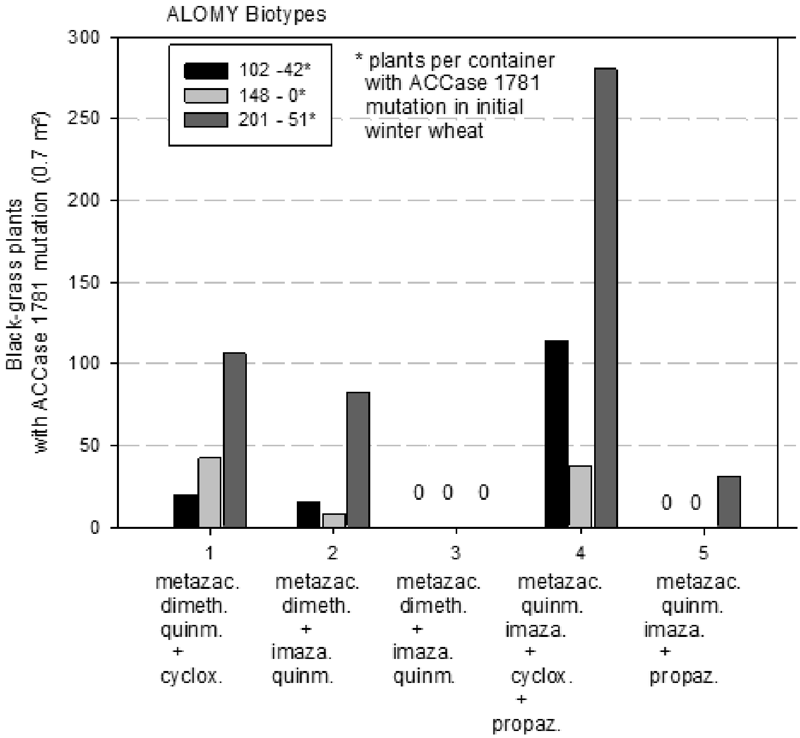

***Fig. 4** ALOMY plants with ACCase TSR 1781 in CL-OSR depending on biotype and herbicide programme.*

Abb. 4 ALOMY Pflanzen mit ACCase TSR im CL-Raps, abhängig von Herkunft und Herbizidapplikationsfolge.

Treatment 3 resulted in no surviving plans for all biotypes, thus no leave samples were collected (Fig. 4). The same was for biotype 102 and 148 after treatment 5. The selection of TSR ALOMY plants due to herbicide strategies differ between the ALOMY biotypes. It depends on the resistance situation on the field and the resistance characterisation of the ALOMY populations which herbicide strategy will be successful. For example treatment 2 (pre-emergence and post-emergence application) reduces TSR-ALOMY plants in biotype 102, while biotype 201 showed increasing numbers of ACCase TSR. Also treatment 4, with three applications timings, did not control biotype 201. The number of ACCase TSR increased in this case dramatically.

Discussion

The strong influence of herbicides on the evolution of herbicide resistance is well known. DÉLYE *et al.* (2007) detected a strong relationship between the frequency of use of ACCase inhibitors and an increasing number of resistant black-grass biotypes. The powerful selective pressure targeting a single gene or a few genes is consequently expected to rapidly increase the frequency of mutation conferring the adapted phenotypes (MENCHARI *et al.,* 2006; MARECHAL *et al.,* 2009; PETIT *et al.,* 2010). This is why we started the investigations of the impact of imazamox containing herbicides on the development of resistance in black-grass. The trial includes three black-grass biotypes with different resistance pattern. Biotype 102, originated from UK reflects with its wide ACCase- and ALS-TSR (+ NTSR) the "typical" British black-grass biotype. Biotype 148, originated from Germany shows a slight ACCase-TSR + NTSR which are also typical for German black-grass. The third biotype 201, originated from France shows a very strong ACCase-TSR at the position 1781 (+ NTSR) and reflects the French black-grass situation. This study shows that the efficacy of the herbicide programmes clearly depends on the resistance pattern of the black-grass biotype on the field. There is no general recommendation of one single herbicide strategy to avoid the risk for further selection of resistant weeds. The good results of the Kerb flo treatment lead to the assumption, that this herbicide could preserve everything. Regarding the optimal treatment conditions in the container trial (for example watering the container after the treatment) it is possible, that we overestimate the efficacy of Kerb flo. In the field situation it is quite often not possible to spray Kerb flo in the winter time because of the inappropriate weather conditions. It is necessary to act before.

The frequent use of ALS-inhibitors in one crop rotation can be problematic if the risk of further selection of resistant weeds will not be regarded. Therefore, it is still necessary to follow the general recommendations of resistance management with all non-chemical aspects, independent if CL-OSR or non CL-OSR is grown.

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