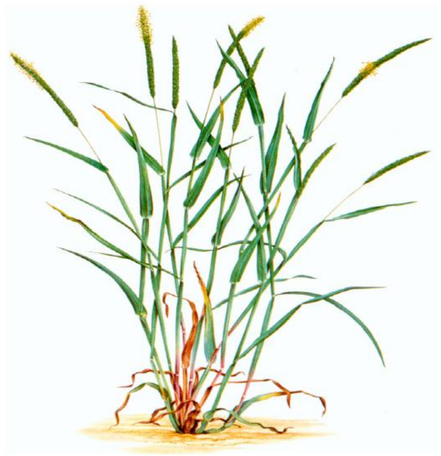
Treatment Influence on Herbicide Resistance Level of Belgian *Alopecurus myosuroides* HUDS. (Black-Grass) Populations



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ABSTRACT

Black-grass is a common grass weed, widely spread in Northern Europe and also in Belgium. For ages, it has been an increasing problem in industrial crops, especially winter cereals. Therefore, farmers started to spray herbicide intensively and soon cases of failure occurred for different molecules and different modes of action. Black-grass populations have been tested in greenhouses to assess the influence of an herbicide treatment as to the resistance level regarding three different herbicides: chlortoluron, fenoxaprop-P and mesosulfuron+iodosulfuron.

Black-grass seeds were collected in field trials in six locations in Belgium, on individuals **which had survived the herbicide treatment**. Each population comes from trial plots, measuring 2 meters wide by 5 meters long and characterized by a single or a combination of products. Herbicides sprayed were isoproturon, flufenacet+diflufenican, ACCase inhibitors and ALS inhibitors. **Seeds were also collected in the untreated plots**. The population present in these last ones corresponds to the former population, before the herbicide selection pressure was applied. In the glasshouse assay, this population was used as the standard population to compare with other populations issued from the same field. The 'R' rating system was set up with this population to assess the evolution of resistance level, year in, year out, using Rothamsted and Peldon populations as reference scale.

Each field population presented different behaviours towards herbicide applied in greenhouses and some cases of resistance can be highlighted. Generally, a reduction of treatment efficiency between field and greenhouse results was clearly visible for the whole of studied active ingredients. Indeed, a distribution shift of the populations towards higher resistance classes could be observed. This is particularly remarkable for active ingredients sharing the same mode of action. For example, it has been found that populations already sprayed with fenoxaprop-P on the field showed a higher resistance level to fenoxaprop-P than to mesosulfuron in the greenhouse test.

MATERIAL AND METHODS

Black-grass seeds were collected in early July 2007 in six winter wheat fields in Belgium well-known for their high infestation level of black-grass (Middelkerke, Slijpe, Pecq, Lisogne, Thy-le-Baudoin and Perwez). On each field a trial was conducted to compare the effects of herbicide treatment (Field treatement FT 0 – FT 6), consisting in spraying different herbicides on small plots measuring 2 meters wide by 8 meters long. Seed collection was carried out on matured ears of black-grass individuals that were present in those parcels, which means that they resisted the applied treatment. Then seeds were transferred into paper bags and stored at 32°C.

- FT 1 : PUMA S EW (fenoxaprop-P 69 g.a.i. ha^{-1})
- FT 2 : TOPIK (clodinafop 40 g.a.i. ha^{-1})
- FT 3 : ATLANTIS WG (mesosulfuron 9 g.a.i. ha-1 + Iodosulfuron 1,8 g.a.i. ha-1)
- FT 4 : CAPRI (pyroxsulam 18,8 g.a.i. ha-1)
- FT 5 : HEROLD (flufenacet 240 g.a.i. ha^{-1} + diflufenican 120 g.a.i. ha^{-1})
- FT 6 : IPFLO SC (isoproturon 1000 g.a.i. ha^{-1})

One glasshouse single dose assay was conducted on those populations according to the protocol published by Moss *et al.* (1998) for the screening "ring test". The populations '*Rothamsted*' and '*Peldon*' were included in the test as respectively susceptible and resistant standards (Moss, 1999). Each population was tested for three treatments + Untreated with four replicates (Glasshouse Treatment GT 0 – GT3). Commercial formulations of the following herbicides were used at field rate:

- GT 1 : mesosulfuron+iodosulfuron (9+1,8 g.a.i. ha^{-1}, 300 $g.ha^{-1}$ ATLANTIS WG, Bayer CropScience+ 1 $l.ha^{-1}$ Adjuvant ACTIROB B);
- GT 2 : fenoxaprop-P (69 g.a.i. ha^{-1}, 1 $l.ha^{-1}$ PUMA S EW Bayer CropScience + 1 $l.ha^{-1}$ Adjuvant ACTIROB B);
- GT 3 : chlortoluron (2500 g.a.i. ha^{-1}, 5 l. ha^{-1} LENTIPUR 500 SC, Nufarm).

Herbicides were applied four weeks after sowing when plants had reached the three-leaf stage (BBCH 13). An experiment sprayer equipped with a 2 meters wide boom, carrying 4 nozzles delivering 250 litres spray solution ha^{-1} was used. After three, four and five weeks, all plants were scored using a visual scale in percentage (0% no destruction, 100% dead, classes 5%) prior to harvest. After six weeks, fresh foliage weights were measured for each pot, and the percentage reduction in fresh weights relative to untreated plants was calculated.

DISCUSSION

Glasshouse assay

- Chlortoluron is included in this test to represent the substituted ureas family. It is rather the isoproturon that is used in agriculture but it is not convenient to use in glasshouse because of its root activity and to the watering system applied. Moreover, the pre-emergence spraying is not feasible in this kind of test.
- Mesosulfuron represents the ALS-inhibitors family in this test. It will be interesting to assess the resistance level to mesosulfuron of a population sprayed with the other ALS inhibitor (pyroxsulam) which has not yet been used in agriculture. It can show if the plants previously exposed to this kind of product can acquire a resistance level to a new but similar active ingredient. It is also a good indicator of the resistance mechanism, enhanced metabolism against the whole herbicide class or target-site resistance aiming only at one kind of molecule.
- Fenoxaprop-P represents the ACCase-inhibitors family and the same comparison can be made between fenoxaprop-P and clodinafop.

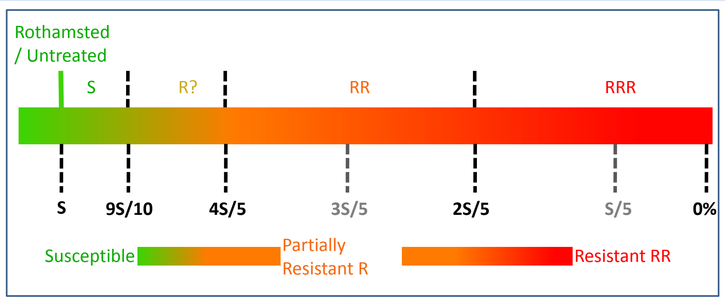


Figure 1 : 'R' rating system (Moss *et al.*, 1999). The 'S' value is based on the fresh foliage weight of the standard population. The classes intervals are calculated with this number.

DISCUSSION

Resistance Level Classification

•The resistance level of each population was assigned using the 'R' rating system (Moss *et al.*, 1999), as shown at Figure 1. The 'S' value is a percentage of destruction, based on the difference between the fresh foliage weight of the untreated (GT 0) and the treated standard population, for each herbicide (GT).

•The classes intervals are calculated with this number, at 90%, 80%, 40 % of 'S' value.

S1 : (Weight Untreated GT 0 – Weight Treated with herbicide 1 GT 1) / Weight Untreated GT 0

S2 : (Weight Untreated GT 0 – Weight Treated with herbicide 2 GT 2) / Weight Untreated GT 0

…

Ex : mesosulfuron (7,65 g – 0,52 g) / 7,65 g = 93,21 % = S1 → Classes : 93,21 ; 83,89 ; 74,56 ; 37,28 ; 0.

fenoxaprop-P (7,98 g – 1,12 g) / 7,98 g = 85,94 % = S2 → Classes : 85,94 ; 77,34 ; 68,75 ; 34,38 ; 0.

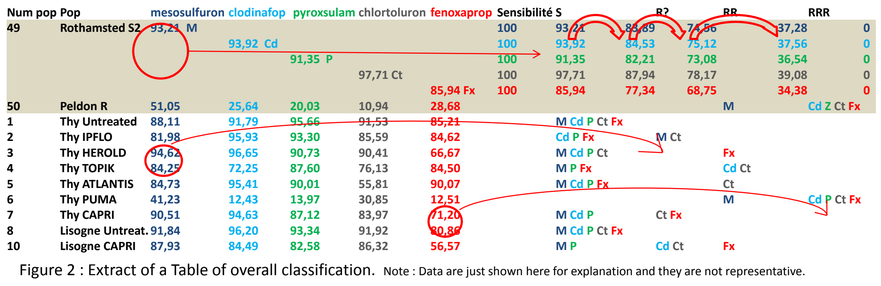
…

•Then for each population and for each herbicide (GT), a percentage of destruction is calculated with the same formula. With this value, we can assign the population to the resistance class of the corresponding herbicide. (See Figure 2)

•At first, the standard population is based on the '*Rothamsted*' standard, to compare the whole of the populations together.

•Then we recalculated the classes using as standard population, the 'Untreated' population of each field location (FT 0). We can assume that this population corresponds to the standard of the location. As those individuals were not exposed to a new selection pressure exerted by herbicide (FT), the population that grew in the field is more or less the same as the former year, and so can be used for comparison. Like that, we can avoid the bias coming from the genetic background of each field, conferring more or less resistance to herbicides.

•For each herbicide treatment (GT) and for each population (corresponding to an herbicide FT), we got a resistance classification. We grouped then each FT between themselves, to assess the herbicide (FT) influence on the resistance level of plants.



Num pop	Pop	mesosulfuron	clodinafop	pyroxsulam	chlortoluron	fenoxaprop	Sensibilité	S		R?		RR		RRR	
49	Rothamsted S2	93,21 M					100	93,21		83,89		74,56		37,28	0
			93,92 Cd				100	93,92		84,53		75,12		37,56	0
				91,35 P			100	91,35		82,21		73,08		36,54	0
					97,71 Ct		100	97,71		87,94		78,17		39,08	0
						85,94 Fx	100	85,94		77,34		68,75		34,38	0
50	Peldon R	51,05	25,64	20,03	10,94	28,68							M	Cd Z Ct Fx	
1	Thy Untreated	88,11	91,79	95,66	91,53	85,21		M Cd P Ct Fx							
2	Thy IPFLO	81,98	95,93	93,30	85,59	84,62		Cd P Fx			M Ct				
3	Thy HEROLD	94,62	96,65	90,73	90,41	66,67		M Cd P Ct					Fx		
4	Thy TOPIK	84,25	72,25	87,60	76,13	84,50		M P Fx					Cd Ct		
5	Thy ATLANTIS	84,73	95,41	90,01	55,81	90,07		M Cd P Fx					Ct		
6	Thy PUMA	41,23	12,43	13,97	30,85	12,51							M	Cd P Ct Fx	
7	Thy CAPRI	90,51	94,63	87,12	83,97	71,20		M Cd P			Ct Fx				
8	Lisogne Untreat.	91,84	96,20	93,34	91,92	80,86		M Cd P Ct Fx							
10	Lisogne CAPRI	87,93	84,49	82,58	86,32	56,57		M P			Cd Ct		Fx		

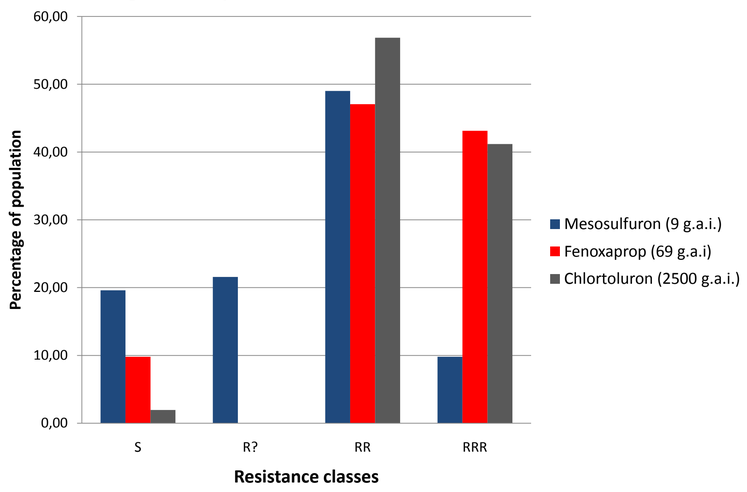
Figure 2 : Extract of a Table of overall classification. Note : Data are just shown here for explanation and they are not representative.

Population N°	Localisation	Field Treatement	Efficacy	X	Y	Z	100	S	R?	RR	RRR	
61	Middelkerke	Untreated	mesosulfuron	92,03				92,03	82,83	73,62	36,81	0
			fenoxaprop		39,82			39,82	35,84	31,86	15,93	0
			chlortoluron			65,91		65,91	59,32	52,73	26,36	0
62	Middelkerke	IPFLO		66,45	13,69	54,42			Ct	M	Fx	
63	Middelkerke	HEROLD		61,71	5,50	55,30			Ct	M	Fx	
64	Middelkerke	TOPIK		50,00	11,11	46,11				M Ct	Fx	
70	Pecq	Untreated	mesosulfuron	97,10				97,10	87,39	77,68	38,84	0
			fenoxaprop		32,04			32,04	28,83	25,63	12,81	0
			chlortoluron			59,91		59,91	53,92	47,93	23,96	0
71	Pecq	IPFLO		66,23	3,09	7,63				M	Fx Ct	
72	Pecq	CAPRI		77,84	40,16	43,35		Fx	M	Ct		
73	Pecq	PUMA		69,88	39,93	31,36		Fx		M Ct		
74	Pecq	ATLANTIS		19,90	1,35	48,19			Ct		M Fx	

Example IPFLO :
1 pop R? to Ct
2 pop RR to M
2 pop RRR to Fx
1 pop RRR to Ct

Figure 3 : Extract of a Table of classification, sorted by location, with the Untreated population as standard.
Note : Data are just shown here for explanation and they are not representative.

Figure 3 : Populations issued from the whole of trial fields



RESULTS

Comparison with '*Rothamsted*' Standard

Comparison with '*Rothamsted*' Standard

Results are presented in percentages of population sorted by resistance classes at Figure 3. Almost every population tested here has a pretty **high resistance level**, mostly RR and RRR.

Mesosulfuron (GT 1) seems to exert a more effective control than chlortoluron and fenoxaprop-P. Mesosulfuron resistance development has been fairly quick since it has only been authorized for six years in Belgium and only for winter wheat, which means one time in a rotation

The mesosulfuron dose used in this test is **9 g.a.i. ha^{-1}** (300 g. ha^{-1} of ATLANTIS WG), which is the common authorized dose in Belgium. In case of resistant black-grass, this dose can be raised up to 15 g.a.i. ha^{-1} (500 g. ha^{-1} of ATLANTIS WG). With such a dose, we can expect a certain amount of highly resistant black-grass to be redistributed in a lower class (R?→ S ; RR → R?). According to the weak percentage of RRR populations, the resistance mechanism involved should be **enhanced metabolism**.

Fenoxaprop (GT 2) and chlortoluron (GT 3) offer only a poor control of these populations. Those fields were chosen for the trials because of their high level of resistance. As there is no intermediate level of resistance to fenoxaprop-P, we can expect some cases of **target-site mutation** of the ACCase gene. This will be confirmed by DNA tests in two fields (Slijpe and Pecq). Nevertheless, the global high level of resistance is certainly also due to **enhanced metabolism**.

Comparison with 'Untreated' Standard

The untreated population (FT 0) of each location is used as a standard to calculate the resistance classes of the 'R' system.

This untreated population figures last year population, as no selection pressure has been exerted on it on the field by an herbicide treatment. As these populations come from trial plots with reduced heterogeneity, and the different treatments sprayed are perfectly known, we can get indications about the resistance level evolution from one year to another.

It is evident that the **treatment modifies the resistance level** since the distribution has generally shifted to the right, towards classes of higher resistance level. If there were no effects of treatment, every population would have the same resistance level as the original population from the former year, and so be sorted in the 'S' class.

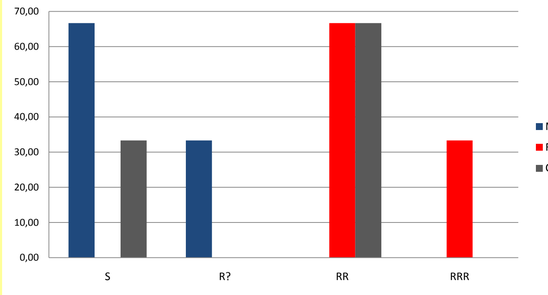
The effect of each treatment is compared between each locations, taking as a reference the untreated population (FT 0) of the same location. (See Table 1 : for example Lisogne 24, Thy-le-Baudoin 32 and Perwez 45 for the fenoxaprop-P).

Graphs presented here show the whole of fields treatments (FT 0), mentioned with by commercial names of the products applied, to avoid confusion with the herbicide tested in the glasshouse assay (GT) and because some of them are constituted of several active ingredients.

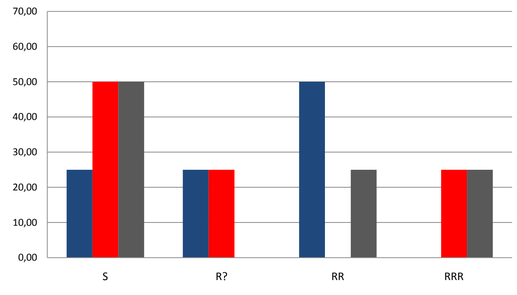
The example of the PUMA S EW (fenoxaprop-P) (FT 1) is fairly representative of the selection pressure effect, leading to a population shift towards higher resistance classes for the same molecule. **Every black-grass individual that survived the PUMA S EW treatment previous year is much more resistant to fenoxaprop-P (GT) than if untreated**. A similar effect can be observed for the chlortoluron. This could lead to think about a multiple resistance to these two molecules, the same mechanism of detoxification could be involved in the resistance. **An application of PUMA S EW on the field keeps a low resistance level to mesosulfuron (GT)**, which means that the population that resists PUMA S EW can easily be controlled by ATLANTIS the next year. It confirms the hypothesis of herbicide rotation with different modes of action (Moss *et al.*, 2007).

For the **TOPIK (clodinafop) (FT 2)**, it is difficult to conclude there is an influence of the treatment, except a slight tendency to select resistance towards mesosulfuron (2/4 ≤ RR). **The effect on fenoxaprop-P is fairly limited (1/4 ≤ RR) though they use the same mode of action**. The resistance mechanism involved might be selective to the molecule and not towards the whole 'FOP' product family.

PUMA S EW (fenoxaprop-P 69 g.a.i. ha^{-1}) (FT 1) 3 Locations

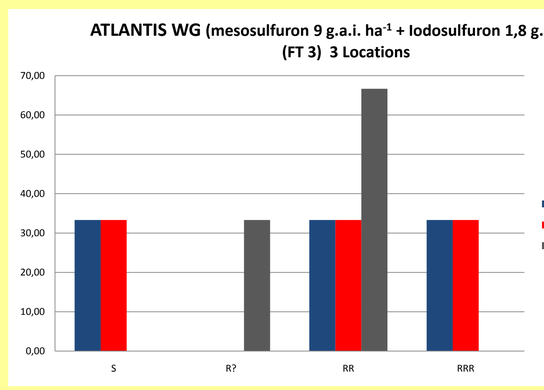


TOPIK (clodinafop 40 g.a.i. ha^{-1}) (FT 2) 4 Locations

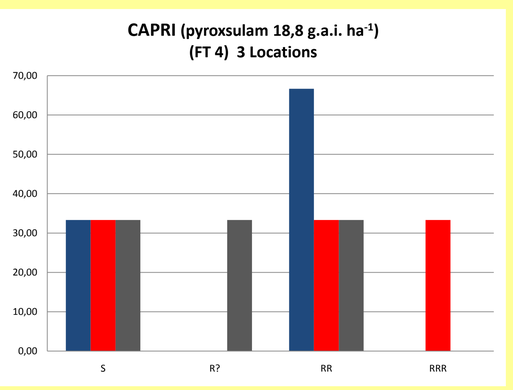


The analysis concerning the ALS-inhibitors, ATLANTIS WG (FT 3) and CAPRI (FT 4) is not really relevant. The distribution between active ingredients is not really contrasted but at least there is a shift to the resistance classes for every one. An effect of ATLANTIS WG and CAPRI on the mesosulfuron sensitivity can be observed in two cases out of three. If it is quite predictable for ATLANTIS WG, it is more important for CAPRI. This new product that has never been used so far is already surpassed by resistant black-grass individuals. The **resistance mechanism towards mesosulfuron also affects this new ALS-inhibitor active ingredient**. Both products select plants for their resistance to fenoxaprop-P (2/3 ≤ RR). Contrary to CAPRI, ATLANTIS WG also increases the resistance level to chlortoluron (2/3 ≤ RR). We could conclude that a multiple resistance has developed towards the three modes of action but the few data available limit the reliability of this test.

ATLANTIS WG (mesosulfuron 9 g.a.i. ha^{-1} + Iodosulfuron 1,8 g.a.i. ha^{-1}) (FT 3) 3 Locations



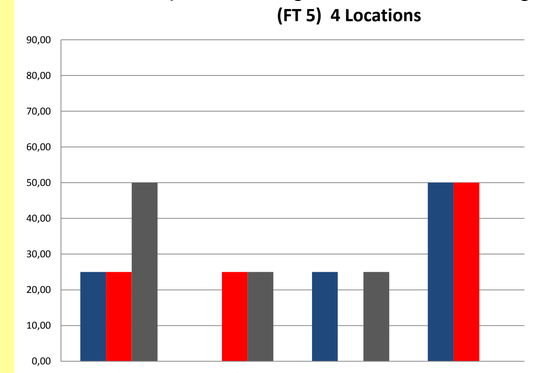
CAPRI (pyroxsulam 18,8 g.a.i. ha^{-1}) (FT 4) 3 Locations



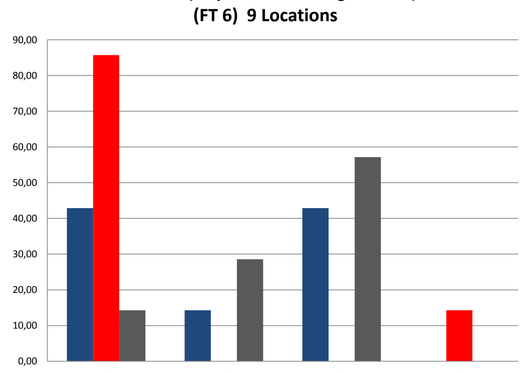
HEROLD is a commercial formulation containing diflufenican and flufenacet (FT 5), two herbicides with other modes of action than those tested in this assay. Nevertheless, we can see a rather strong selection towards fenoxaprop-P and mesosulfuron, but only a weak one towards chlortoluron.

IPFLO SC (isoproturon) (FT 6) does not exert any selection pressure on fenoxaprop-P, except one population that could present a multiple resistance. There is a slight effect on mesosulfuron. Otherwise, IPFLO SC selects for resistance to chlortoluron, but not until RRR class, which could confirm that there is no target-site mutation involved.

HEROLD (flufenacet 240 g.a.i. ha^{-1} + diflufenican 120 g.a.i. ha^{-1}) (FT 5) 4 Locations



IPFLO SC (isoproturon 1000 g.a.i. ha^{-1}) (FT 6) 9 Locations



CONCLUSION

A global **negative impact on the treatment efficiency** is clearly visible for the whole studied active ingredients. Indeed, a distribution shift of the populations towards higher resistance classes can be observed. This is particularly remarkable for active ingredients sharing the same mode of action. Spraying a 'FOP', ACCase-inhibitor will select amongst the original population individuals presenting a resistance to fenoxaprop-P, just as spraying a new ALS-inhibitor molecule can lead to the apparition of mesosulfuron-resistant black-grass. Some cases of cross-resistance have also been reported.

This study confirms that **the herbicide choice is particularly important to avoid recurrent selection of mutant individuals in the field**. Moreover, with the high multiplying coefficient of the black-grass (± 500 seeds per plant (Moss, 1985)), the spreading of the resistance can be rather quick. Further studies are in progress to evaluate the propagation of resistant individuals within a parcel.

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