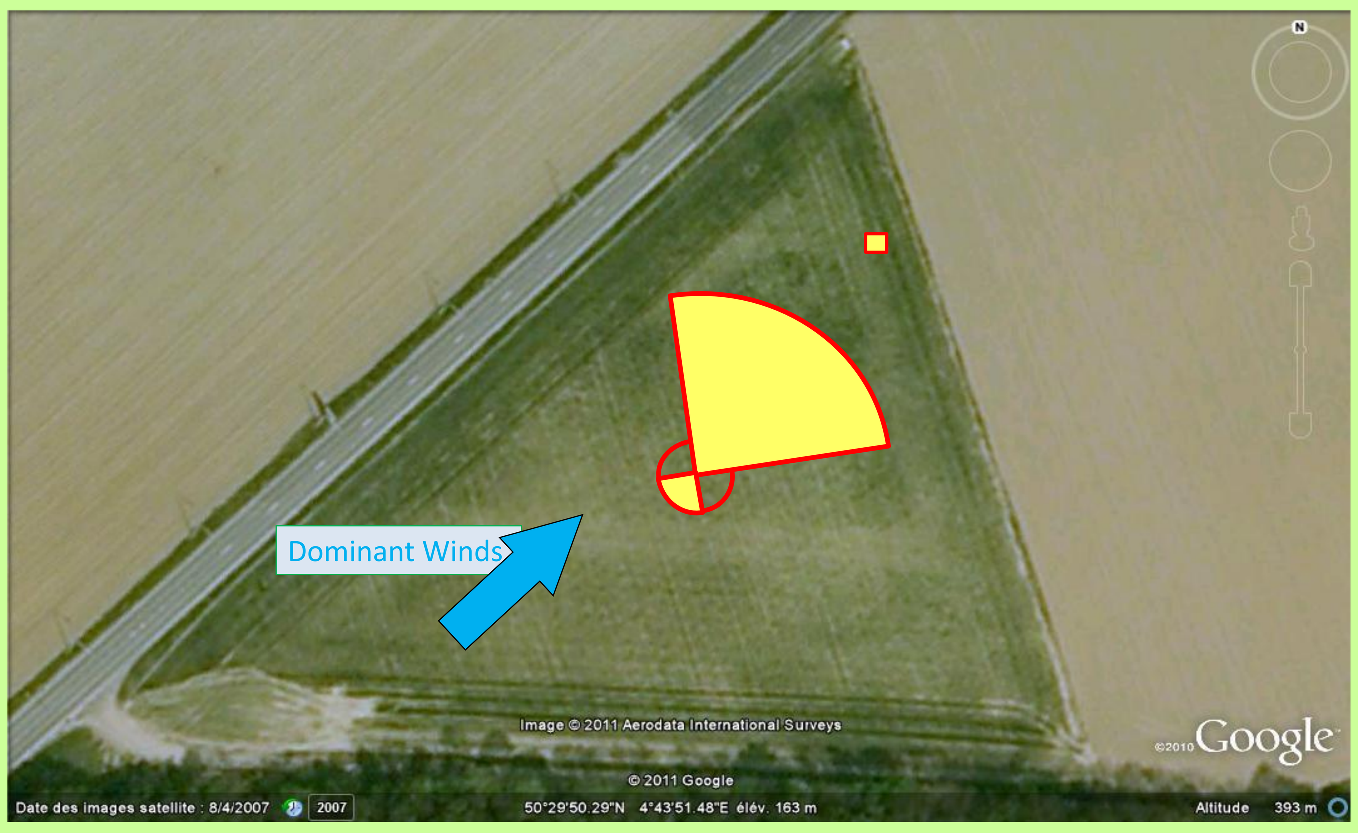
Pollen Dispersal Abilities of ALS Target-Site Resistant Black-grass (*Alopecurus myosuroides* HUDS.)

Maréchal Pierre-Yves *[(1)], François Henriet[(2)], Françoise Vancutsem[(1)] et Bernard Bodson[(1)]
[(1)] University of Liège – Gembloux Agro Bio Tech Crop Production in Temperate Regions Unit / Passage des Déportés, 2, B-5030 Gembloux Belgium
[(2)] Walloon Centre of Agronomical Research – Life Science Department (D1) Plant Protection and Ecotoxicology Unit (U4) / Rue du Bordia, 11, B-5030 Gembloux Belgium
email : pymarechal@doct.ulg.ac.be



Picture I : Aerial view of the experimental design fitted on the field. Note upwind and downwind areas and the single plot on the 45° ray, at 128m from the introduction point.

SUMMARY

Target-Site Resistant (TSR) black-grass individuals were introduced in a field, at different growing stages to be synchronised with autochthonous population. Patches of susceptible black-grass were created by setting protective canvas on few areas (from 0,5 up to 32m²), during herbicide spraying and they were removed afterwards. Those patches were disposed along rays starting from the introduction point at distances from 2 to 128 meters. In those patches, local black-grass grew regularly and was able to receive some pollen from the resistant individuals, located in the centered emitting area. The herbicide field treatment (mesosulfuron+iodosulfuron : 15+3 g.a.i.ha-1) was efficient, confirming that the local population is susceptible to ALS inhibitors, was preceded by another herbicide treatment targeting only broad-leaves weeds, performed on the entire field, including patches, such as the rest of cultural practices such as fertilization, and fungicide and insecticide treatment. All seeds were harvested in each patches separately and tested in glasshouse. Seed samples were sprayed with sulfometuron, herbicide active ingredient known not to be affected by non-target site resistance, in a way to detect the percentage of resistant black-grass that can be engendered after the introduction of TSR individuals and to determine the distance resistant pollen can cover. DNA analyses were performed on surviving plants to confirm the presence of the mutation.

Key words: Black-grass, Target-Site Resistance (TSR), AcetoLactateSynthase (ALS), Pollen, Dispersal

WHAT DISTANCE CAN BLACK-GRASS RESISTANT POLLEN COVER WITHIN A WINTER WHEAT FIELD ?

INTRODUCTION

A farm field located in the south-western part of Belgium (Quévy), has been identified presenting a high level of target-site resistance (TSR) to ALS inhibitors, due to the W574 mutation, whereas no ALS-based treatment was ever sprayed on it. Hypothesis is that this mutation was fixed in the population a long-time ago and that the first ALS inhibitor herbicide application, hence modifying the environment and inducing a new selection pressure, only revealed the presence of that specific mutation, conferring in this case a higher fitness to resistant individuals. Nevertheless, this field constitutes a huge reservoir of resistant pollen that could be flown away to neighbouring fields. But how far can black-grass resistant pollen travel within a field?

MATERIAL AND METHOD

Resistant black-grass individuals were introduced within a winter wheat sown field, belonging to the experimental farm of Gembloux Agro Bio Tech. It was chosen also for its relative isolation towards other potential uncontrolled black-grass around the introduction point (See Picture I). On one side, in the opposite direction of dominant winds, the motorway, bordered by tall trees and thick bushes, represents a physical barrier to avoid pollen dispersal. The introduction point was sufficiently distant from these trees to avoid any turbulence, which could have modified the dispersal pattern. On the other side, downstream from dominant winds, neighbouring fields were sown with sugar beet and potatoes. In these spring crops, black-grass is usually well-controlled and moreover there is a delay in flowering time between black-grass winter and spring populations, avoiding any contamination at longer distance.

In April 2010, a total of 10 homozygote resistant plants were transplanted in the field. Seeds were issued from crossings that we carried out for other experiments (Marechal *et al.*, 2010), between two resistant homozygotes (mutation W574) individuals (named later as RR, in opposition with RS for resistant heterozygotes), themselves issued from Quévy field. Plants were grown in glasshouses at different stages to ensure the flowering synchronisation with the autochthonous black-grass population. DNA analyses (dCAPS, Délye & Boucansaud, 2008) were performed to control the genotype.

To avoid a complete "contamination" of the field, **small patches of autochthonous susceptible black-grass were created by setting a protective canvas on a few areas**, during herbicide spraying and removed afterwards. Those patches were disposed along rays centred on the introduction point at angles of (0, 15, 30, 45, 60, 75, 90, 135, 180, 225, 270 and 315°) at several distances (2, 4, 8, 16, 32, 64 and 128 meters from the centre). See Figure I. Plots' size was gradually doubled in order to intercept the same area proportion at each distance. Covering half of the arc's area, the sheets measured from 0,5m² at 2m up to 32m² at 128m. Black-grass average density of 10 plants/m² ensures the **presence in each plot of autochthonous black-grass individuals, able to receive some pollen** from the resistant individuals, located in the emitting area.

All the seeds were harvested in each plot, once a week from June 29 till July 19. **A sample of seeds of each plot** of the 45° ray (from -8m till +128m) , from two collecting dates, was **grown in glasshouses then sprayed with sulfometuron-methyl** (200 g.a.i.ha-1), which is known not to be affected by metabolic resistance, thus revealing only target-site resistant plants (Marshall & Moss, 2008). **DNA analyses were performed on surviving plants to confirm the presence of the mutation, which means that resistant pollen was carried up to the plot.**

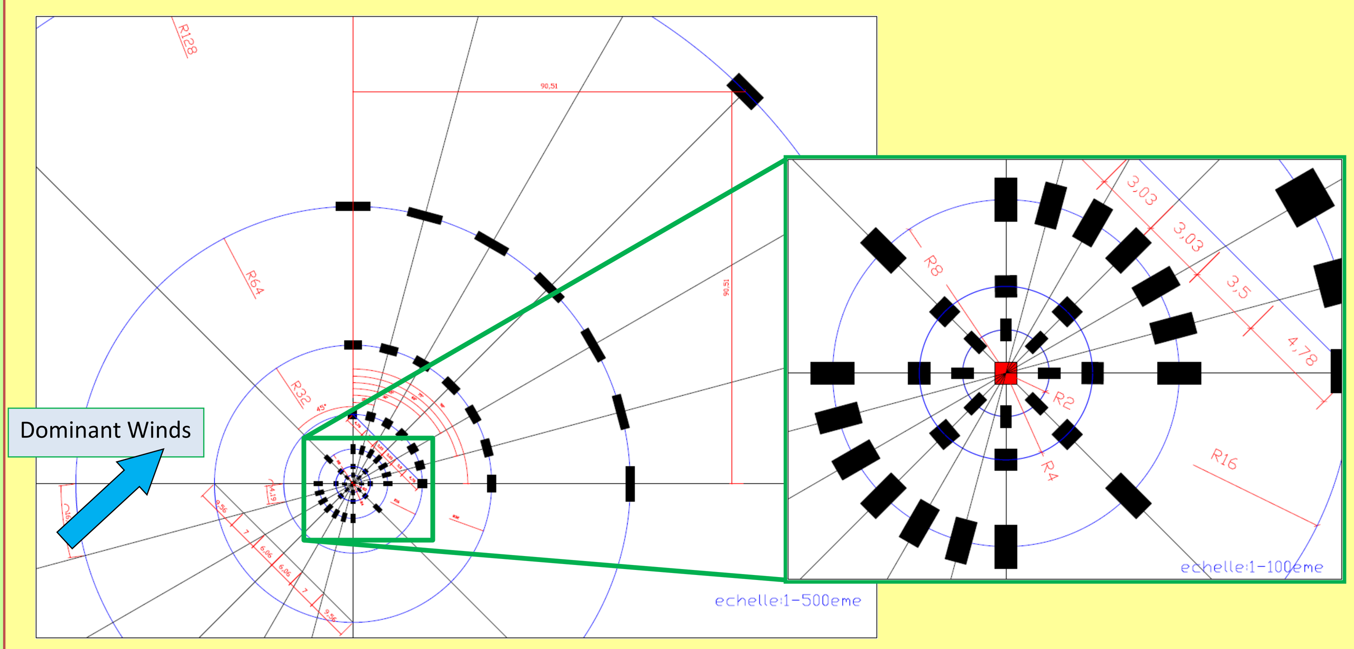


Figure I : Experimental design implemented on the field thanks to protective canvas disposed on different rays at 2, 4, 8, 16, 32, 64 and 128m away from the introduction point. Global scheme and zoom on the central part. The introduction point is figured in red. The orientation was fitted with dominant winds (SO → NE).

Picture II : Implementation of the experimental design on the field. Central point with introduced resistant individuals (10 plants) and the rays starting in each directions. Each stick represents the central point of the plot, except from the central ones, figuring introduced plants.

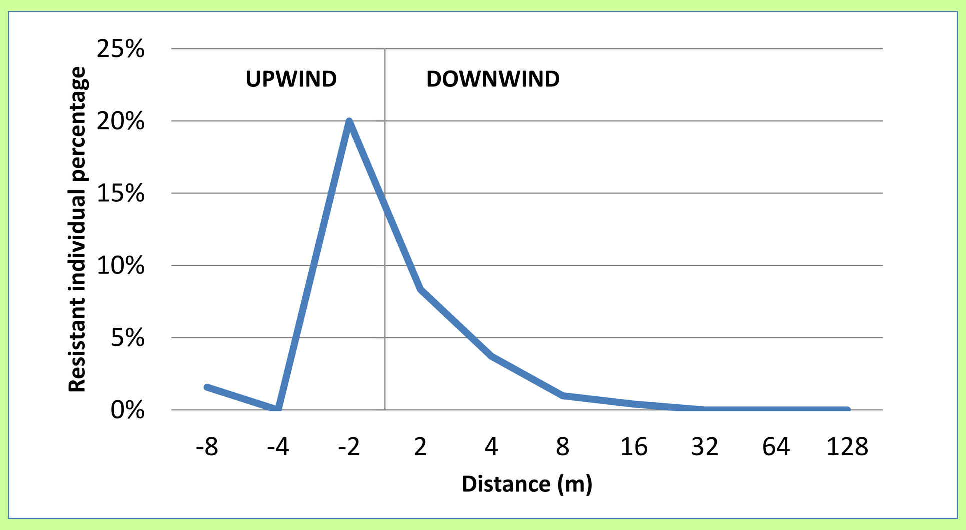


Figure II : Resistance dispersal pattern: Resistant individual percentage in the offspring at different distances (Upwind and Downwind, on the 45° ray)

NO RESISTANCE FOUND AFTER 16 METERS REGULAR DECREASE WITH THE DISTANCE

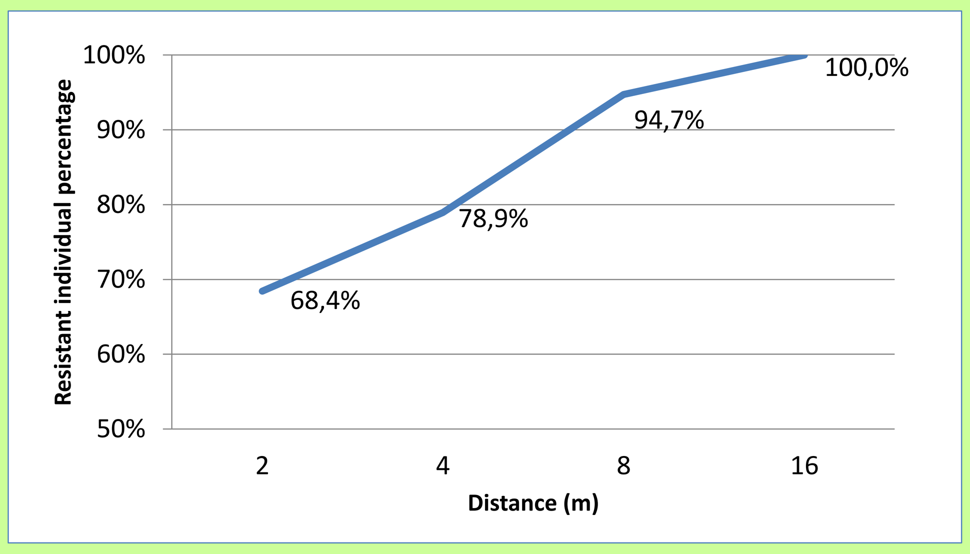


Figure III : Cumulated resistance percentage on the 45°-ray (considering upwind and downwind contribution)

95% OF POLLEN DISPERSAL OCCURS WITHIN AN 8 METERS RADIUS IN THIS DIRECTION

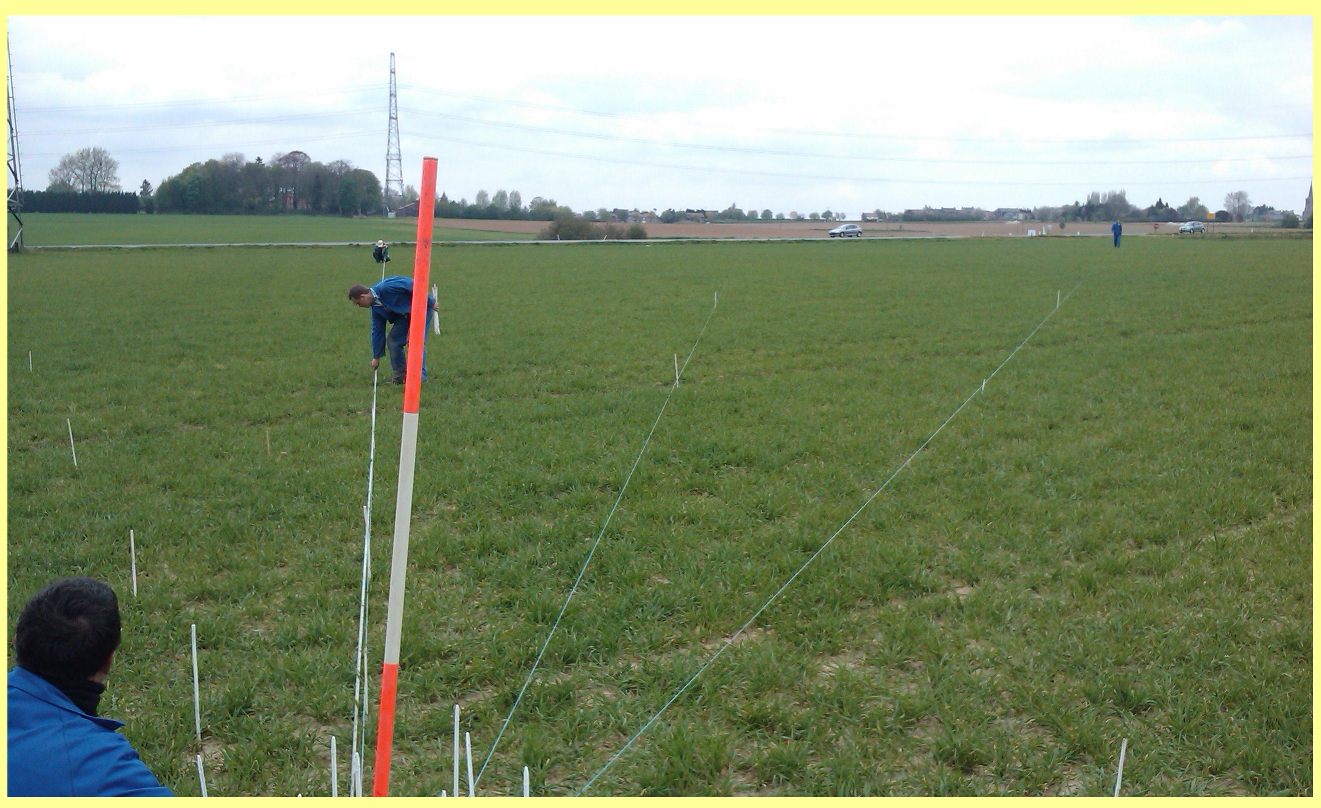
CONCLUSIONS

Compared to results presented by Petersen *et al.* (2010), who showed a maximal distance for outcrossing from the initial plot of 4 meters, in controlled conditions, we highlight here that **resistant pollen can cover at least 16 meters.**

This dispersal distance is really important to tackle the problem of resistance spread, within a field but also at a larger scale, within the landscape. However, with a small size resistance "inoculum" (10 plants on 1m²), 95% of the dispersal occurs within an 8 meters radius in this 45° ray, so the long distance impact seems to be limited.

Knowing that **the dispersal can be rather quick in the immediate neighbouring** (up to 20 per cent of the offspring in 2meters radius has acquired the resistance gene), it is primordial to nip it in the bud and avoid any long term resistance spread.

Samples collected in other rays have to be tested in a way to assess whether the resistance distribution pattern is different in other directions.



Picture III : Global view of the field, from the introduction point to the different rays. Each stick represents the central point of the plot.

RESULTS AND DISCUSSION

Seeds were tested in glasshouses in a way to determine the distance and the percentage of resistant black-grass that can be engendered, after the introduction of a small group of TSR individuals, and the maximal distance at which the resistance gene can be detected in the offspring.

A total amount of 2500 seeds were tested (See Table I). Proportion increased with the distance from the introduction point, in order to increase the probability of finding resistant plants, and depending on the seed sample size. For example, there were rather few autochthonous plants in the "-4m" plot (only 23 seeds available) and none managed to catch resistant pollen.

20% of tested individuals from 2 meters upwind plot carried the resistance gene, whereas only 8,3% in the 2 meters downwind plot. This can be explained by the fact that there had been North-West winds during flowering time (opposite to regular dominant winds). Another fact is that the autochthonous black-grass density was weaker in the "-2m" plot, and thus cross-pollination between individuals located within the plot was limited.

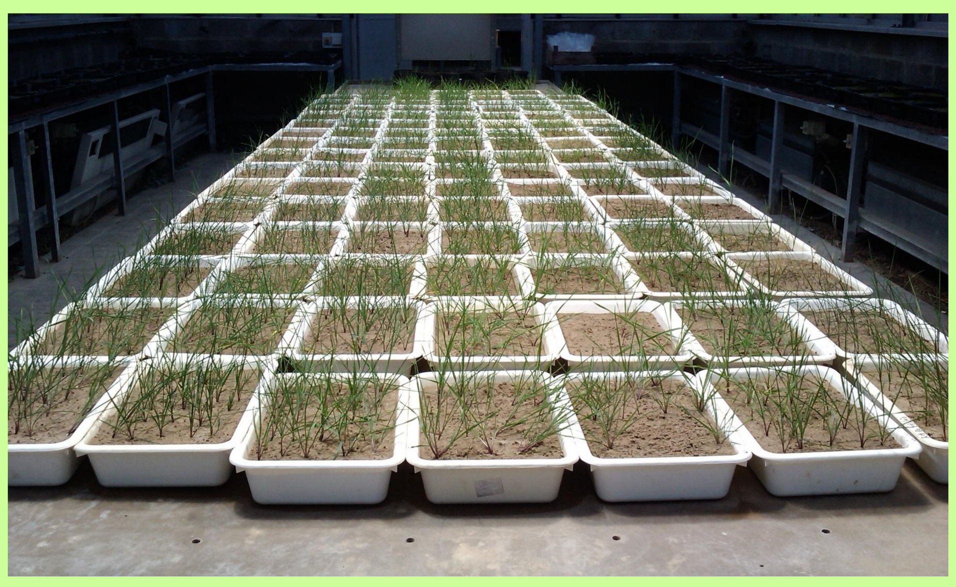
At the "4m" plot, a little less than 4% of resistant black-grass were detected. At 8 meters radius, as much upwind as downwind, around 1% of resistance was identified. This should also be due to opposite winds.

At last, **one individual carries the resistance gene at 16 meters.**
The resistance dispersal pattern is showed at Figure II, and except for the "-4m" plot, where no resistant was found, the shape of the curve looks normal and the decrease is constant with the distance (8,3% - 3,7 % - 1,0% - 0,4% at respectively 2, 4, 8 and 16 meters from the introduction point.

13 individuals on 100 tested, coming from the 2 meters radius, showed resistance, which means that nearly 70% of the resistant pollen stays in the immediate neighbouring of mother-plant (See Figure III). 95% of pollen dispersal occurs within 8 meters radius and no resistance has been found after 16 meters from the introduction point.

Table I : Glasshouse assay results, for two collecting dates on the 45°-Ray (Up- and Downwind)

Distance (m)		Number of resistant individual	Number of tested individal	Resistant individual Percentage	Resistance distribution	Cumulated resistance distribution
-8	UPWIND	2	128	**1,6%**	10,5%	10,5%
-4		0	23	**0,0%**	0,0%	10,5%
-2		8	40	**20,0%**	42,1%	52,6%
2	DOWNWIND	5	60	**8,3%**	26,3%	78,9%
4		2	54	**3,7%**	10,5%	89,5%
8		1	102	**1,0%**	5,3%	94,7%
16		1	246	**0,4%**	5,3%	100,0%
32		0	296	**0,0%**	0,0%	100,0%
64		0	739	**0,0%**	0,0%	100,0%
128		0	750	**0,0%**	0,0%	100,0%
Total		**19**	**2438**	**0,8%**	**100,0%**	



Picture IV : Glasshouse assay. View of the trays used for sulfometuron-methyl spraying test.

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