Detection of ACCase Target-Site Resistant <i>Alopecurus myosuroides</i> Huds. (Black-Grass) in Belgian populations

P.-Y. MARECHAL<sup>1</sup>, F.HENRIET<sup>2</sup> and B. BODSON<sup>1</sup>

<sup>1</sup>Gembloux Agricultural University (UgAsa), Unité de Phytotechnie des Régions Tempérées, Passage des Déportés 2, BE-5303 Gembloux, Belgium

<sup>2</sup>Wallonia Agro-Food Chain Research Centre (OBA-W), Pesticides Research Department, Rue du Bord 11, 18, BE-5303 Gembloux, Belgium

Corresponding author: marechal.p-y@fgas.be

**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. The first case of resistance in Belgium was reported in 1996 by Robert Bulcke’s Team (Elen et al., 1996). Yet the resistance mechanism was not specified. Since then, no more information was published about Belgium, while research continued in the United Kingdom and in France. Moreover, during the last decade, progress in molecular biology allowed to highlight the mechanism of target-site resistance. A simple PCR method allows to detect the mutation conferring resistance to herbicides.

After two years of resistance monitoring in Belgium, mostly in the Wallon part, some populations have been clearly identified as highly resistant to ACCase inhibitor. These populations have been tested by molecular biology so as to detect the single nucleotide polymorphism (SNP) involved in this case. The method employed was the Polymerase Chain Reaction Allele Specific Assay (PCR-AS: Delay, 2002) for the mutation Bef-175-E in 86 susceptible samples from 48 fields.

**RESULTS**

The first PCR was performed to amplify the fragments flanking the mutation, with the same primers as for the RBA, but only the forward primer in order to amplify all ACCase positive DNA. Each DNA sample was put in reaction with the 1–5 primers (30µl) to amplify the wide-type A allele fragment and in another PCR tube, with the 21–25 (30µl) primers (15µl only A or C mutant allele, respectively). An agar gel electrophoresis was carried out to separate the amplicons. For both A/A samples, this reaction did not give any step, which confirms that they are susceptible. For the DNA samples, 8 amplicons were obtained, extruded from the gel and resolved. 13–14F A – 478, 4 – 68F – 5’– 7F – 7F; 7 – 670F, A – 30 P A.

Every further handling is detailed in the previous section. The results obtained for the sequencing with two replicates per fragment are presented at Figure 3 (wild type fragments) and at Figure 4 for Bef-175-E with no C allele. PCR allele-specific amplification gave the same results, shown on the gel presented at Figure 2.

**CONCLUSION**

We can say that Delays’ method, described in his paper (2002 A), has been successfully transposed to our lab in Gembloux. The Bef-175 mutator of the ACCase gene has been discovered in three Belgian Blackgrass populations. Those populations are located in three different locations: the Walloon “Thameslands” (South Western) and South Center part of the Wallon Region. Both subpopulations of the 534 A allele have been detected. Only heterozygote mutant have been discovered in those samples.