Introduction
Over the last 40 years there has been a dramatic increase in the frequency and diversity of herbicide-resistant weed biotypes, which poses a substantial threat to the sustainability of agriculture both locally and globally. Modern agriculture couples the management of invasive weed species with enhancing crop yields through the intensive use of herbicides. Many of the most problematic weeds including black-grass, have now evolved resistance to multiple herbicides with various modes of action. This project focusses on identifying markers of Multiple Herbicide Resistant (MHR) in the weed black-grass.

Modern agriculture couples the management of invasive weed species with enhancing crop yields through the intensive use of herbicides. Unfortunately, as a result of continuous use and a lack of variety in the modes of action (MoA) the frequency and diversity of weed biotypes expressing evolved resistance to one or more herbicides has dramatically increased. The growing persistence of grass weeds amongst arable crops has indicated a strong negative impact on the sustainability of agriculture both at local and global level, with crop yield losses of up to 50%.

Currently, the greatest concern facing agriculturalists globally is MHR, which is currently found in many of the most problematic weeds including black-grass (Alopecurus myosuroides). The data surrounding the resistant mechanisms a plant utilises to survive is vast, although gaining a more coherent understanding is vital in order to maintain and optimize crop yields for future generations.

What is AmGSTF1?
• In 1999, it was identified that the independent MHR black-grass population biotype “Peldon” constitutively expressed a protein which belonged to a plant specific phi class glutathione transferase which plays a role in the detoxification of xenobiotics, this protein was later termed AmGSTF1.1
• AmGSTF1 has high glutathione peroxidase (GPOX) activity reducing toxic organic hydroperoxidases to less reactive alcohols.
• Although poorly understood, in common with multiple-drug resistance (MDR) in tumors, MHR is associated with an enhanced ability to detoxify xenobiotics.

Identification of Biomarkers: New anti-AmGSTF1-serum
• One potential biomarker of MHR in black-grass is the phi class glutathione transferase AmGSTF1. Previously the antibody utilised in screening was raised to ZmGSTF1.2
• Essential to generate a new antisera which was more specific for potential use as a diagnostic kit therefore, synthesised from the cDNA encoding AmGSTF1 from MHR “Peldon” black-grass plants.
• Having cloned the cDNA it was expressed in E.coli as a strep II tagged protein for affinity purification, prior to rabbit immunization.
• Test antisera samples were then tested for their immuno-selectivity on crude black-grass protein extracts and resolved by SDS-PAGE prior to western blotting.

Figure 1. Dendrogram depicting AmGSTF1 and other closely related phi class glutathione transferases.

Aims
• This project focusses on identifying biomarkers of MHR in the weed black-grass which have the potential to be developed into molecular research tools to study the evolution of MHR in the field.
• The approach adopted was that of “omics,” to identify biomarkers which could be developed into a series of portable diagnostic platforms based on DNA, immuno or small molecule recognition technologies.

Transcriptomics
• Gene expression
• Microarrays

Proteomics
• Protein identification
• LC/MS

Metabolomics
• Metabolomic profiling
• LC/MS
• GC/MS

Identification of Biomarkers
• New anti-AmGSTF1-serum
• Use in diagnostic kits
• POC (point-of-care test)
• Microarrays

Future Work
• Following its primary validation as a useful biomarker of MHR, work on developing anti-AmGSTF1-serum based diagnostic devices for the identification of resistant weed populations in the field is currently in progress.

Figure 2. AmGSTF1 crystal structure

Figure 3. Western blot analysis depicting the result of the immuno-selectivity on crude black-grass protein extracts. In MHR polypeptides of 25,24 and 22kDa detected strongly.

Figure 4. Western blot analysis depicting the results of extracts from ten different black-grass populations following exposure to anti-AmGSTF1-serum. Polypeptides of 25,24 and 22kDa detected.

References