

‘Multiple Herbicide Resistance in Grass Weeds: from Genes to AgroEcosystems’

Case for support Part 1A. Previous track-record of applicants

All references in part 1 may be found in the cv annexes of the PIs

This project brings together the expertise in the molecular biology and biochemistry of herbicide selectivity and tolerance (Robert Edwards; **RE**, University of York), epigenetics (Dr Louise Jones, University of York), weed ecology, evolution, management and herbicide resistance (Paul Neve; **PN**, University of Warwick), evolutionary ecology and modelling (Rob Freckleton; **RF**, Dylan Childs; **DZC**, University of Sheffield and Jarrod Hadfield; **JDH**, University of Oxford) and environmental impact assessment (Ken Norris; **KN**, University of Reading). The goal of the consortium is to take our latest understanding of the molecular basis of herbicide resistance in grass weeds and use this information to elaborate the population and evolutionary dynamics of selection for resistance under field conditions, ultimately designing novel control practices to mitigate its evolution and spread. In particular, the project will focus on populations of the problem weed black-grass (*Alopecurus myosuroides*) which have acquired resistance to multiple classes of graminicides, through enhanced herbicide metabolism. Showing many parallels with multidrug resistance in animals, we have termed this multi-herbicide resistance, or MHR. The project represents a truly interdisciplinary weed science programme taking advances in basic plant biochemistry through to applied plant physiology, field ecology, quantitative genetics, evolutionary biology, modelling and applied land use research. This ‘agri-systems’ approach will take basic bioscience through to practical translation in dealing with one of the most significant challenges to sustainable cereal production in the UK. Importantly, all the groups involved in the programme have well established reputations in their respective fields of expertise.

The **York group** are internationally known for their work on the detoxification of synthetic compounds and natural products in plants. In the course of a BBSRC funded research development fellowship (2003-08), **RE** developed the concept of the xenome to describe the collective working of the metabolic biosystem responsible for controlling the biological activity of small molecules in plants through biotransformation and transport. Work on the xenome in the **RE** lab has extended from Arabidopsis, to the major cereal crops and associated weeds, including wild grasses. Work programmes have retained a strong applied focus with a series of projects funded directly by the chemical sector, as well as through BBSRC responsive mode and initiative grants, industrial collaborative/ LINK awards and TSB technology programme awards. The long term objective of the xenome project is to selectively manipulate plant metabolic networks responsible for controlling the biological activity of natural products and synthetic compounds for applications in fine chemicals production, sustainable crop protection and phytoremediation. The **RE** group has a long history of studying the functions of xenome enzymes in herbicide metabolism and selectivity in crops and weeds and were the first to characterize the phi (F) class glutathione transferase, termed *AmGSTF1*, associated with MHR in black-grass. These observations led to BBSRC grants BBS/B/09201 and BB/G006474/1 which have proved to be spectacularly successful. In addition to defining the MHR biochemical phenotype in black-grass, a regulatory role for *AmGSTF1* in herbicide resistance was determined through a combination of molecular genetic and chemical intervention approaches. The papers describing these studies have recently been submitted for publication, though the respective findings can be found in a UK patent on the engineering of multiple herbicide resistance in plants (GB 0717882.3) and the US/EU patent WO2009034396 detailing the use of mechanism-based inhibitors to counteract herbicide resistance in grass weeds. In preparative work for the current proposal, the **RE** group have also performed detailed transcriptomic and metabolomic analyses on MHR Vs. wild type sensitive

(WTS) black-grass and annual rye-grass (*Lolium rigidum*). These studies now form the basis of developing a research tool kit of biochemical and genetic probes to investigate the evolution of MHR in black-grass in the field. The development of these diagnostics tools will benefit from RE's joint post as the Chief Scientist at the Food and Environment Research Agency (Fera), which is a world leader in field based detection technologies for plant health applications.

The work at York will be carried out in the Centre for Novel Agricultural Products (CNAP) which received major funding from BIS in 2012 to establish a fast-track molecular breeding facility in support of the Biorenewables Development Centre. As a consequence the consortium will have access to the latest facilities in next generation sequencing and high throughput genetic marker analysis. The work will be undertaken by named researcher, Dr Melissa Brazier-Hicks who has extensive experience of xenome biochemistry, genomics through working with a variety of weeds and crops and has direct experience of the methods and approaches to be adopted (see CV). Another aspect of the work which will require additional support is in informatics, based on the large scale transcriptomic analyses to be performed in **Obj 1.1**. This work will be undertaken by a Dr Yi Li who has been central in developing the informatics capability at CNAP (see CV). In addition, Dr Louise Jones will contribute expertise in epigenetics for the analysis of DNA methylation and histone modifications of genes induced by MHR. She has over 15 years experience in the field of epigenetics including expertise relevant to the project in the analysis of trans-generational events and stress-induced epigenome change (see CV).

The **School of Life Sciences (SLS)** at the University of Warwick has major research themes in plant and crop science, focused on global food security. The University has recently completed the construction of a £5m phytobiology research facility providing state-of-the-art controlled environment and glasshouse facilities for plant-based research at Warwick. Dr Paul Neve (PN) has international experience in the ecology, evolution and management of herbicide resistance. The overarching aim of his work has been to design and evaluate proactive management strategies for the mitigation of resistance evolution. Research highlights have included the first demonstration that high levels of genetic variation for MHR-based resistance are present in grass weed populations (*Lolium rigidum*) prior to selection. This standing variation can be rapidly selected and recombined giving rise to highly resistant phenotypes over 2-3 generations at reduced and standard herbicide application rates. This paradigm-shifting research provided the first evidence of the importance of standing variation versus *de novo* mutation in the dynamics of resistance evolution. PN has developed novel methods for the determination of fitness costs associated with herbicide resistance. He co-authored the first report of a fitness cost associated with MHR. PN has led international research efforts to develop simulation modelling approaches for the management of glyphosate resistance. This research has integrated molecular understanding of resistance mechanisms with ecological, evolutionary and agronomic insight to deliver novel resistance management solutions that have been adopted by growers. Modelling work and other strands of research in the PN group are supported by the agrichemical industry, directly (Syngenta, Monsanto) and in the form of BBSRC CASE studentships (Bayer and Dow). A current large DEFRA-funded project is investigating the ecology of UK populations of black-grass, generating data and knowledge with direct application in this project. PN has ongoing international collaborative herbicide resistance research with colleagues in the USA, Australia, Argentina, France, Germany and Spain.

The **Department of Animal and Plant Sciences (APS)** at the University of Sheffield is one of the largest in the UK devoted to the study of whole organism biology. The department has strength in integrative plant sciences, particularly the scaling from genotypes and individual level phenomena to populations, communities and ecosystems. **Professor Rob Freckleton** is an

ecologist focusing on modelling populations and communities of animals and plants with a view to understanding and predicting the impacts of environmental change in the widest sense. He has worked on plant population modelling for 19 years, with a focus on weed populations. Research conducted as part of his PhD and post-doctoral demonstrated the possibilities for modelling to contribute to understanding and predicting the effects of changing management on weed populations and biodiversity. His models were used to predict the effects of GM crops on farmland biodiversity as well as to suggest how GM crops may be used to promote biodiversity. In recent research the emphasis has been on developing methods that allow weed populations to be monitored and modeled at scales ranging from field to farm scales. In a previous RELU-funded project this approach was used to monitor common weeds on up to 50 farms for 4 years, with 10 fields mapped per farm down to a resolution of 20 x 20m. These data, including extensive information on populations of black-grass, have been used in population modeling, as well as uncovering the relationships between socio-economics, management and weed abundance.

The research of **Dylan Childs** has focused on the use of data-driven modelling to understand the environmental and ecological drivers of population dynamics and natural selection. He is supported by a NERC Advanced Fellowship, following-on from a 3-year NERC Fellowship. Early research examined the evidence for evolutionary bet-hedging in natural plant populations, using state-of-the-art approaches from biodemography and evolutionary game theory. This work has emphasised the need to consider the joint dynamics of life-history traits and demography to fully understand short-term responses to environmental change. Working at longer temporal scales, he has also examined the role of demographic structure and ecological feedback in shaping the life-histories of natural populations. The theoretical and analytical methods that have been developed in this work form the basis for the modeling we propose here.

The **Department of Zoology** at the University of Oxford is one of the largest and most diverse in the UK, with a long tradition of ecological and evolutionary research. **Jarrod Hadfield** is a population geneticist and statistician, currently a Royal Society University Research Fellow, working on theoretical and empirical aspects of selection and inheritance in the presence of family interactions. He has published widely in the field of quantitative genetics including on aspects of theory, methodological development and empirical application. In acknowledgment of this work he was invited in 2012 as a plenary speaker at the international quantitative genetic conference. In this proposal, Hadfield will contribute expertise in the design and analysis of the breeding experiments, and in linking the quantitative genetic data to evolutionary models of selection and evolutionary response.

The **Centre for Agri-Environmental Research (CAER)** at the University of Reading focuses on understanding the relationships between land-use, biodiversity and ecosystem services in agro-ecosystems in the UK, Europe and Overseas. This work is increasingly being linked to valuation studies that explore the economic consequences of land management options. **Professor Ken Norris** is a biodiversity scientist who works on how populations, communities and ecosystems respond to environmental change. Over recent years, this work has included developing novel approaches for assessing the impact of agricultural land-use change on UK and European biodiversity. This work has appeared in high quality journals such as *Science*. In this proposal, Norris will lead the work that explores the possible environmental and economic consequences of land-use change associated with herbicide resistance and its management. This work will utilize a range of approaches and tools developed previously.

All the PIs have track records in working in large and successful consortia. In addition to his role as head of profession to 500 scientists at Fera, **RE** was co-director and founder of the Centre for Bioactive Chemistry at Durham University, now the Biophysical Sciences Institute. **RE** also led the RC-funded synthetic biology national network Synthetic Plant Products for Industry from 2008-11.

RF has recently completed a prestigious 5-year Leverhulme Trust funded Research Leadership Award in which a series of study sites were established on a latitudinal transect along the Atlantic coast from Portugal to the UK. This formed the basis for a series of experiments on population and community interactions, as well as metabolomic studies and evolutionary modeling. Both **DZC** and **JDH** have worked extensively with established consortia focused on long-term monitoring of model animal populations (e.g Soay sheep of St Kilda), and have established successful national and international collaborations with world leaders in mathematical population biology. **PN** worked for six years as part of a collaborative, multidisciplinary research team that brought together plant scientists, ecologists, agronomists, modellers and economists to study all aspects of the biology and management of herbicide resistance. Since returning to the UK, he has established international collaborations within industry and academics. **KN** has worked extensively with UK-based consortia focusing on understanding the environmental risks of agricultural change and the causes of biodiversity loss; and with international consortia focusing on the links between agriculture, biodiversity and ecosystem services in tropical forest-agriculture ecosystems. Since 2005, **KN** has led CAER at the University of Reading, a research centre that has now established a leading reputation for agro-ecological research. Since 2009, **KN** has also acted as the Biodiversity Theme Leader for NERC, which involves working with the research community to develop programmes that address various issues relating to biodiversity loss and its consequences. To date, NERC has invested c£30M in these programmes.

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Case for support Part 1B

Statement on Data Sharing

Molecular Data

All sequence and metabolomic data, including raw data files, will be stored on a secure server in the Centre for Novel Agricultural Products. All processed sequence data arising from the programme will be put into the public domain into open databases at the earliest opportunity following publication and no later than the conclusion of the programme. Raw data files will be freely accessible on request following publication for the duration of the grant to ensure quality assurance.

Ecological and Quantitative Genetic Data

A number of major international journals in ecology and evolutionary biology have made public data archiving a mandatory part of the publication process, and this is now becoming routine for projects such as the one we propose. Our intention will be to fully archive the data from this project in public archives as part of the publication process. Specifically we will:

- (i) Archive the weed survey data in the UK data archive. The data from our RELU project have been accepted for archiving in the UK data archive and submitted. We will add the new data to this archive to add to these data.
- (ii) DataDryad will be used as a second archive, more routinely accessed by international ecologists and evolutionary biologists. The combined set of ecological, Quantitative Genetic and resistance data will be archived here.
- (iii) We will publish a data paper in the journal Ecology to highlight the data, as well as to act as a citeable source for the data.

Our data will be suitably anonymized to ensure that individual farmers or farms cannot be publically identified from the archives. Our anonymization will be approved by the University of Sheffield Ethics Committee prior to submission.

We have so far archived our data in plain text files, along with text files containing explanatory information, and this format was accepted by the UK data archive. We will do the same for the data generated by this project.

Models

The models we develop will be developed and made publically available on github, a widely used platform for disseminating code and software. This can be used freely for publically available projects.

Reporting

The accessibility of data arising from the programme will be a standing item for annual meetings with the stakeholder group to monitor the effectiveness of the consortium’s activities in up-dating the wider community on dissemination activities.

Case for support part 2. 'Multiple Herbicide Resistance in Grass Weeds: from Genes to AgroEcosystems

Background- Food Security and the importance of herbicide resistance in weeds

Food security is currently one of the most pressing issues for both science and society. In recent years food production has been affected by climate change, economic pressure and also increased concerns about the environmental impacts of farming methods. There is pressure to maximize the efficiency and outputs of farming. However, at the same time there is social and political pressure to minimize agronomic inputs where possible and there is a need for balancing production with the other ecosystem functions of modern agriculture. One of the most significant inputs into cereal production is the use of herbicides to control weeds². In Northern Europe, the repeated use of graminicides to control wild grasses has led to the large scale evolution of herbicide resistance in weeds, notably black-grass *Alopecurus myosuroides* which can often result in local infestations^{3,4}. Black-grass is the major weed threat in arable rotations in the UK with herbicides applied to an estimated 1 million hectares per annum for black-grass control. Resistance to one or more herbicides is estimated to occur on 80% of farms and recent projections suggest that up to 20% of wheat production in the UK is under threat due to inability to control black-grass. As part of a wider trend in agriculture, evolved resistance to herbicides has been confirmed in 210 plant species⁵ and has become one of the greatest threats to sustainable crop protection in northern Europe. Ironically, resistance threats are escalating at a time when regulation is removing herbicide modes of action from the management armoury and reducing permissible herbicide application rates^{6,7}. Furthermore, there have been no new herbicide modes of action discovered for over 20 years⁸⁻¹⁰.

The evolution and molecular basis of herbicide resistance

There are two common modes of resistance in grass weeds. 1) Target-site resistance (TSR) is endowed by point mutations in herbicide target enzymes, and usually gives rise to tolerance to all herbicides sharing that mode of action. 2) Non target-site resistance (hereinafter, multiple herbicide resistance, or MHR), encompasses a range of possible mechanisms which often give rise to resistance to a broad range of herbicide modes of action. Often, these mechanisms are based on the enhanced metabolic detoxification of crop protection chemistries, though mechanisms relating to herbicide sequestration and reduced translocation of herbicide have also been documented¹¹. While TSR can be managed through the rotation of herbicides with differing modes of action, MHR is much more difficult to counteract, as it acts irrespective of herbicide chemistry. MHR also appears to be more widespread than TSR. In recent work in support of this proposal, Neve and co-workers found elevated levels of herbicide metabolism (in comparison to a susceptible control population), in over 90% of 92 field-collected black-grass populations from the UK. To date, the majority of research on herbicide resistance has focused on TSR and our current lack of understanding of MHR is hampering attempts to develop sustainable resistance management protocols¹².

MHR is most likely inherited in a polygenic manner^{12,13}, evolving via the co-ordinated and constitutive up-regulation of a number of stress-related metabolic and signalling pathways¹⁴. Recent transcriptome experiments conducted in the Edwards lab have confirmed that MHR is underpinned by a large number of genes being constitutively up-regulated (Fig. 1), notably those associated with xenobiotic detoxification, abiotic stress response and gene regulation (transcription factors). The data shown refers to the perturbation in gene expression determined in MHR Vs. wild-type herbicide sensitive (WTS) annual rye-grass (*Lolium rigidum*), with the results helping explain the strong association between resistance to multiple classes of herbicides and enhanced detoxification in this species¹⁴. However, Fig. 1 illustrates that the MHR genotype also encompasses other plant responses linked to exposure to biotic (infection) and abiotic (environmental) stress¹⁵.

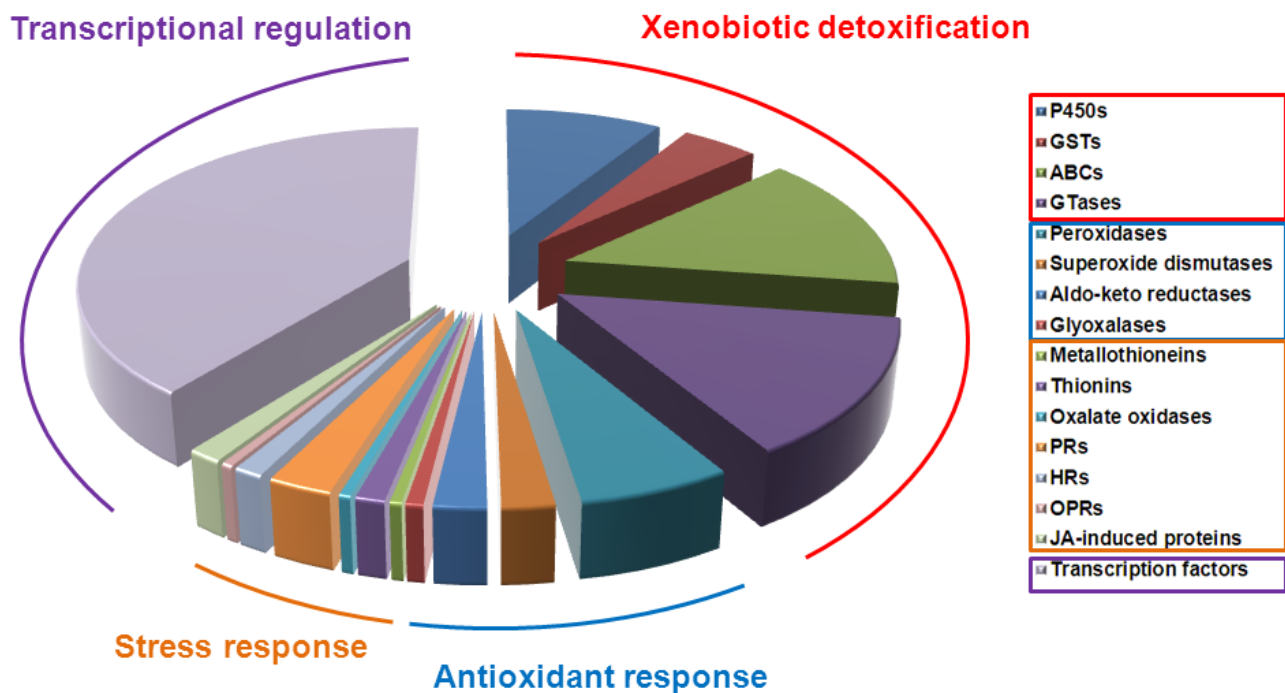


Fig. 1. Pie chart showing relative abundance of the most relevant gene classes identified in the 454 transcriptome data from *L. rigidum* MHR populations¹⁶.

In support of this proposal, similar whole plant transcriptomic studies have been performed in MHR Vs. WTS black-grass and have shown that resistance in this weed shows some fundamental differences to that determined in annual rye-grass. In particular, whereas xenobiotic detoxification shows a major enhancement in MHR rye-grass, only a relatively small number of genes linked to herbicide metabolism are induced by MHR in black-grass (Fig. 2).

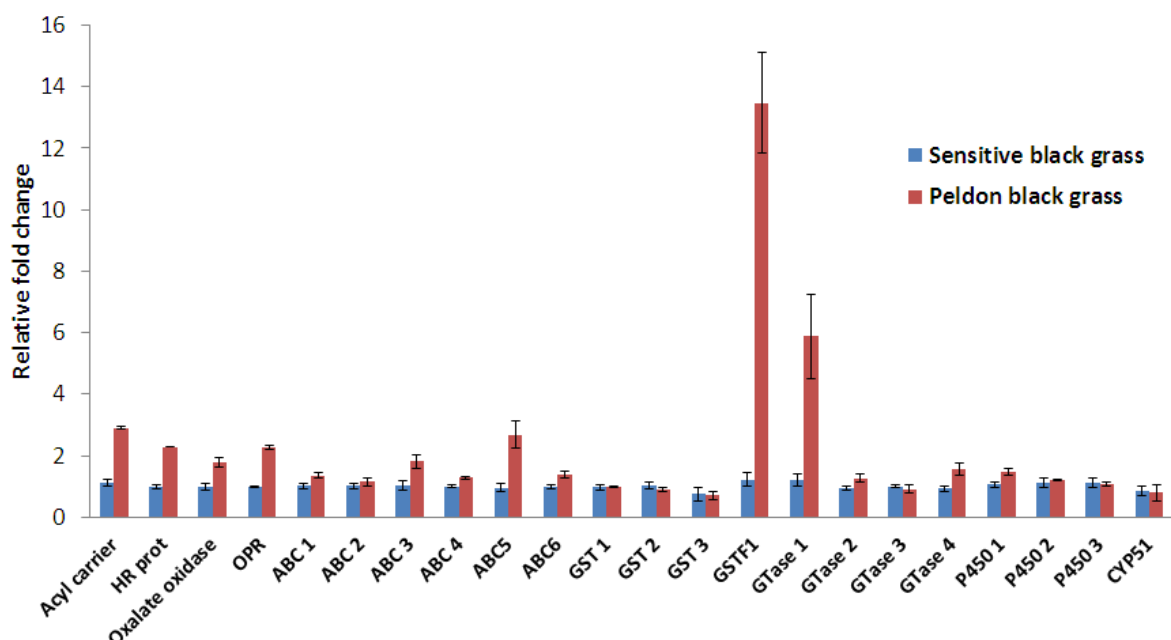


Fig. 2. Expression of genes linked to xenobiotic detoxification in MHR (Peldon) black-grass compared with WTS weeds as determined by qPCR. (ABC= ATP-binding cassette transporter, GST= glutathione transferase, GTase =family 1 glycosyltransferase, P450/ CYP= cytochrome P450 mixed function oxidase).

The major up-regulated gene (Fig. 2), was identified as a phi-class (F) glutathione transferase (GST) termed *AmGSTF1*. Multiple minor-sequence variants of *AmGSTF1* were subsequently

identified, suggesting the presence of multiple gene products, with the respective enzyme activity showing a 20-fold enhancement in activity in MHR Vs. WTS black-grass¹⁷. Intriguingly, the expression of *AmGSTF1* in transgenic *Arabidopsis* under the control of the 35S-promoter conferred partial resistance to herbicides and enhanced antioxidant, xenobiotic and secondary metabolism¹⁸. Since similar biochemical changes are also associated with MHR in black-grass, this does suggest that *AmGSTF1* plays a major role in conferring MHR in black-grass. However, the transcriptome studies also show the induction of other detoxification and stress-related genes (Fig. 2), demonstrating the activation of additional protective responses. In addition the enhanced expression of *AmGSTF1* is subject to complex regulation. For example, in WTS black-grass *AmGSTF1* is known to be transiently induced by osmotic and temperature mediated stress, as well as by exposure to herbicides and partner safeners^{15,17}. On removal of the stress treatment, GST expression in plants normally returns to basal levels¹⁹. In MHR black-grass, *AmGSTF1* is constitutively up-regulated in the absence of imposed chemical or abiotic stress. By way of determining mechanism, in support of this application we have already determined that this enhanced expression is not due to further gene amplification of the multiple *AmGSTF1* genes encoded in the diploid black-grass genome. The logical conclusion therefore is that the normally transiently inducible *AmGSTF1* genes are constitutively activated in MHR plants. Since MHR is a polygenic trait^{12,13}, our working hypothesis is that this type of resistance is caused by the repeated selection for signalling pathways which cumulatively activate the expression of *AmGSTF1* along with the other genes induced by MHR. In the case of *AmGSTF1*, the complex stress inducibility of the respective super-family, whereby exposure to chemicals, abiotic stress and infection lead to the activation of gene family members by apparently independent pathways^{19,20}, provides an ideal frame-work for the conjunction of multiple individual signalling pathways in promoting one cumulative resistance mechanism (= MHR). Two mechanisms could be invoked here. Namely, the repeated selection of natural black-grass variants showing the enhanced expression of MHR associated genes, or acquisition of key resistance traits through epigenetic mechanisms. Based on the currently available evidence, the former selection mechanism would seem more probable in driving evolution in a genetically diverse weed population. However, in recent years, epigenetic changes driving heritable stress tolerance in plants have also been identified²¹, and the potential for such roles in the evolution of herbicide resistance identified in a small number of cases¹¹. Leading on from the cross induction of *AmGSTF1* by both herbicide selection and abiotic stress treatments¹⁵, we also need to understand MHR in the light of other stress responses and the effect this has on black-grass physiology, in terms of diverting resources away from growth resulting in reduced fecundity, or competitive ability⁹.

In evolutionary terms there is therefore a fundamental difference between MHR and TSR. TSR is a qualitative trait, i.e. individuals within a population may be categorised as 'resistant' or 'susceptible' based on the presence of a mutation in a protein targeted by a herbicide (most herbicide resistance traits are dominant at commercial application rates). MHR on the other hand is a quantitative trait, in which variation is underpinned by several loci and environmental factors. To understand the emergence of MHR we therefore need to consider how changes at several loci will, in aggregate, cause a change in the degree of resistance rather than focussing on the evolution of an allele that confers absolute resistance. This issue can be addressed by obtaining measures of population-level genetic variation in the LD₅₀ of plants exposed to different herbicides. High levels of genetic variation in MHR may exist in annual weeds: under selection, particularly at reduced herbicide application rates (as are now advocated in Europe), recent work has demonstrated that selection via the application of sub-lethal doses of herbicides leads to very rapid evolution of metabolic resistance in annual weeds^{1,13}. In annual rye-grass (*Lolium rigidum*) it has been shown that only 3 generations of direct selection are needed to yield marked increases in resistance to the graminicide diclofop-methyl (Fig. 3)¹. Consequently, low rates of herbicide application are predicted to promote the evolution of metabolic resistance. On the other hand, high rates of application of herbicide are more likely to promote target site resistance²². One suggestion is that the evolution of the two forms of resistance are linked. During the initial stages of herbicide application, particularly where low herbicides doses are used, evolution of resistance may occur via selection acting on standing genetic variation in MHR and abiotic stress tolerance. This will lead to increases in the

resistance phenotype, increased plant survival and larger population sizes. Larger population sizes will ultimately increase the probability that novel TSR mutations with lower pleiotropic costs will arise and fix⁹. Under this scenario, there may be no simple optimal strategy: whatever doses of herbicides are used, selection will favour one form of resistance or another, and in the long term the evolution of target site resistance is inevitable.

A greater understanding of the subtle interactions between mechanisms of resistance, population size, life history trade-offs and selection intensity (herbicide dose) and wider management could provide a step-change in herbicide resistance management. This can only be achieved through the integrated systems approach proposed here that combines molecular biology, genetics, ecology, evolutionary biology, weed science, agronomy, and modelling.

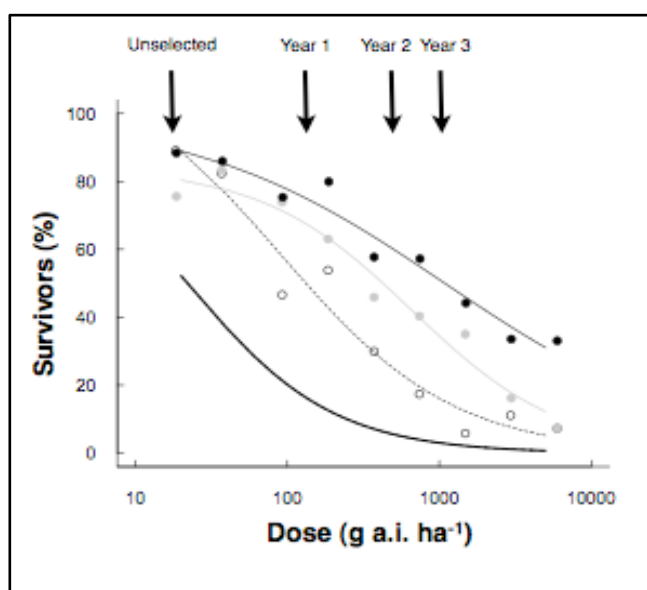


Fig. 3. Selection for resistance over 3 years in an experimental population of *Lolium rigidum*. The assay for resistance involves growing seedlings and recording survivorship under a range of herbicide doses. The thick black line shows the baseline response. The population was subject to selection for 1 year (dotted line, open points), 2 years (grey line, grey points) and 3 years (black line, filled points). Populations were initially selected by subjecting to 10% of the recommended rate of herbicide application (37.5 g a.i. ha⁻¹). The arrows show the shift in the point of 50% survival over the 3 years of selection. Data taken from Neve & Powles (2005)¹. Evolved changes of this magnitude have been documented in several species of weeds using similar methods.

Making long-term and large-scale predictions

The integration of approaches to make system-scale predictions presents a number of challenges. One practical issue is to be able to measure the extent of resistance and types (MHR Vs. TSR) in field populations. This not only allows the extent of the resistance problem to be documented, but also the characterisation of the component populations. In this project, we propose to develop a diagnostic toolkit for all classes of herbicide resistance that will allow us to quantitatively determine related traits in weed populations in the field. In the case of MHR, a major challenge in predicting the rate of evolution of such a quantitative trait is that there may be a complex relationship between the genotype and phenotype because of the multiple genes involved. In this case quantitative genetics offers a useful alternative approach that has been widely applied in plant²³ and animal breeding²⁴. A very general result is that the rate of evolutionary change is proportional to the additive genetic variance of a trait²⁴. In order to predict evolutionary change we therefore need to be able to predict the additive genetic variance and how it might change during the course of evolution. For Mendelian traits the additive genetic variance and its dynamics can be predicted if the causal alleles have been identified and their effects characterized²⁵. For quantitative traits - where many loci may be involved - predicting the additive genetic variance from what is known about the molecular details is exceptionally challenging²⁶. Given this we will estimate the additive genetic variance by comparing the degree of similarity between different types of relative - a well used method that far out performs current molecular methods²⁷. Moreover, although the additive genetic variance is expected to change rapidly during the course of evolution for Mendelian traits, for quantitative traits it is expected to change very slowly. Consequently, the estimates we make in unselected populations are likely to hold in populations undergoing selection.

One of the fundamental tenets of evolutionary biology and quantitative genetics is that of trade-offs: it is generally difficult, if not impossible, for an organism to change one aspect of its biology in order to increase its fitness without incurring a cost. In the case of metabolic herbicide resistance,

increases in metabolism are likely to have consequences for growth and competitiveness. These costs can be quantified by measuring the genetic correlation between resistance and other life-history traits. Such costs are known to exist; for example, metabolic resistance to diclofop-methyl in *L. rigidum* is associated with reductions in whole plant fitness^{9,28}. Although this cost will be more than offset by the advantages of increased survival in the presence of herbicides, understanding the magnitude and basis of such costs will offer some insight into the possible ways resistance can be countered. For example, growing more competitive crops (earlier emerging, taller, higher density) offers the potential for a non-chemical approach that can be used when resistance has developed. Conversely, if fitness costs are negligible then management would be better targeted at reducing the rate of evolution of resistance by changing herbicide management practice. We also need to understand the relationship between MHR and abiotic stress tolerance. If adverse environmental conditions promote the incidence of MHR this will also need to be factored in when proposing integrated weed control strategies.

In order to generate a full understanding of the evolution of herbicide resistance, we need to be able to link population genetic and evolutionary processes with data on abundance, distributions and management of weeds, from within and between population scales. Population modelling offers a route to do this, using techniques that allow us to incorporate genetics and evolutionary trade-offs within ecological models. The framework for modelling the ecology of annual weeds is well understood, and models have been built for a range of species, including species such as black-grass for which herbicide resistance is a problem²⁹. In recent work the Sheffield group have developed monitoring and modelling techniques that permit the generation of large data on sets on the population dynamics of weeds at scales from single fields to whole regions. In this project it is proposed to develop eco-evolutionary models that link together molecular data with the results of experiments designed to measure the additive genetic variance of traits and their trade-offs. These models will then be used to predict the evolution of resistance in the field, and help direct mitigation strategies.

Wider context: why resistance matters beyond field management

The primary role of agriculture is obviously to produce food. However, it is now recognized that agriculture provides a number of other functions beyond this, which have both economic and environmental significance. For example inputs such as fertilizers and agrochemicals are potential pollutants and their benefits in terms of increasing yields have to be offset against potential environmental effects, such as pollution in run-off and non-target effects. At European and national scales, the biodiversity of species living within farmland is a political concern, with large sums of money being spent on conservation. The importance of recognizing that agricultural landscapes are multifunctional should not be underestimated when developing new technology and management recommendations. In the UK the most high profile example of this is the case of GM technology: following a move to introduce GM herbicide tolerant (GMHT) crops, possible environmental concerns led to large scale environmental impact trials, with the conclusion that these crops could harm biodiversity. Largely for this reason these crops were not licensed for use in the UK, despite the possible yield and management benefits of using them.

In this project we will account for wider environmental impacts by combining our models for the evolution of resistance with tools for forecasting wider impacts on the environment. We will therefore anticipate the possible costs and benefits in environmental terms of different management strategies, in addition to evaluating their effectiveness in managing resistance and weed populations.

Project objectives and deliverables

This project will use interdisciplinary basic science to address the following key questions underpinning the evolution of herbicide resistance in black-grass, with the goal of directly applying the outputs of this research as impacts which direct more effective control strategies in the field.

1. What are the molecular mechanisms that underpin the evolution of herbicide resistance in black-grass?
 - What are the genotypes driving MHR and what is the level of variation?

- Does an understanding of MHR genotypes tell us about the evolutionary origins?
- Can we use this information to derive molecular diagnostic kits for resistance ?
- What is the relationship between herbicide resistance and stress response pathways and the potential for epigenetic-driven inheritance of MHR?

Impact: The development of real-time diagnostics which can be used in the field to detect and characterize herbicide resistance mechanisms in black-grass.

2. What is the extent of the herbicide resistance problem in UK black-grass populations and what impacts is resistance having on black-grass populations and crop yields?

- What is the frequency of MHR and TSR at the scale of whole farms and field?
- Can resistance problems and extents be rapidly assessed using molecular diagnostic kits?
- What management practices are associated with resistance, especially MHR ?

Impact: A detailed resistance audit for the extent and distribution of resistance to the major herbicide modes of action in black-grass at a regional scale.

3. What are the genetic, and ecological factors that promote and constrain the emergence of herbicide resistance?

- How much genetic variation for MHR segregates in populations of black-grass?
- Do these variants also affect life-history and reproductive traits?
- What is the rate of evolution of MHR to different herbicides?

Impact: Practical information on the effectiveness of different herbicide resistance management strategies.

4. How can applied evolutionary models be used to manage herbicide resistance?

- How fast does MHR evolve under different control strategies ?
- Does the evolution of MHR contribute to the emergence of TSR ?
- How do (genetic) correlations among life-history traits and resistance influence the evolution of MHR?

Impact: A suite of models which can be used to predict the emergence of herbicide resistance in black-grass and the effectiveness of different control measures.

5. What are the economic and environmental consequences of novel weed and resistance management strategies?

Impact: Management recommendations, together with an analysis of their agronomic, economic and environmental consequences.

Project management

This is a large and diverse project and as such effective management and transfer of information and research materials between the partners will be vital for its effective delivery from the outset. The project is organized into 5 large work packages, each of which are led by one of the partners, but all of which involve multiple investigators so as to derive the maximum benefit from this multidisciplinary consortium. A **Gantt chart** showing the organization of these work programmes is attached as an annex. A key component of the project will be the efficient sharing of plant materials, research results and modeling and monitoring data. Black-grass seeds and plant material will be managed and disseminated centrally through the Neve group at Warwick. Each population will be assigned a unique code and undergo preliminary genotyping at York, prior to resistance audit assessment (Warwick). This information will then be collated centrally at Sheffield through a custom relational database, set up for the duration of the project and accessible to the consortium members. As agreed by the consortium, the project and the published outputs of the project will be maintained in a publically accessible portal maintained through the industrial sponsor, the Home Grown Cereals Authority (HGCA) to allow rapid dissemination of the results of the study to the farming community and other stakeholders. Overall the project will be managed through quarterly progress meetings hosted (and chaired) in rotation by the four partner organizations. The post-doctoral researchers employed on the project will meet more regularly (via

Skype when possible) and will be supported to work between labs to become familiar with essential technologies; for example in the application of the molecular diagnostics technologies developed at York. At each meeting of the PIs, actions will be agreed and a log maintained, with progress against the project objectives monitored. The secretariat for the consortium will be provided through a part-time secretary based at York, who will maintain an actions log, organize meetings and ensure the public portal of the website is updated. In addition to the consortium members, the project will also engage with a stakeholders group drawn from the international weed science community, UK policy owners of pesticide usage, the levy boards, farming community and NGOs.

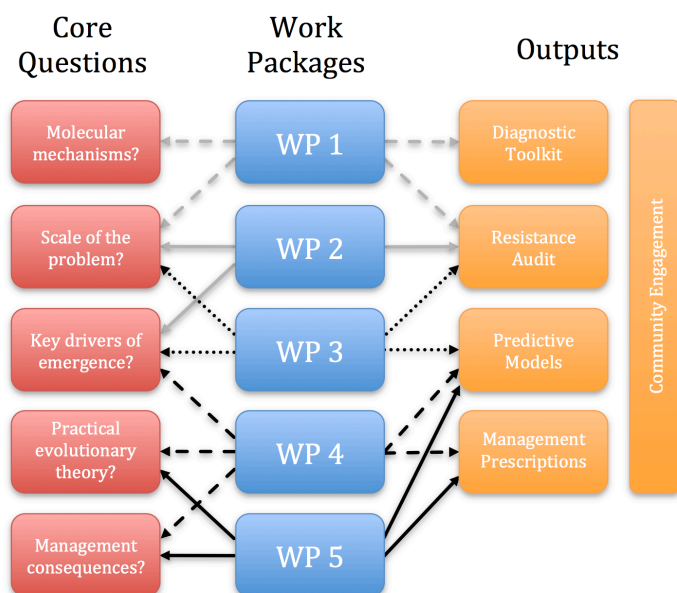


Fig. 4. A schematic diagram showing the projects core research questions and key outputs and the mechanism by which these will be delivered via multidisciplinary work packages. Arrows (formatted to distinguish each work package) show how each work package addresses the core questions and outputs of the project.

The PI will take personal responsibility for the effective governance of both the progress meetings and effective communication with the stakeholder group (see Impact Statement). As described in part 1 of the application, this is an experienced consortium with good prior evidence of both leadership and delivery of outputs through joint working from its members.

Work programme

Work package 1. *Molecular mechanisms underpinning the evolution of MHR in black-grass.* (Rob Edwards and Louise Jones, University of York and Paul Neve, University of Warwick).

This work-package will address the following:

- 1.1 What is the level of variation in the MHR genotype and phenotype in different resistant black-grass populations and what can this tell us about the evolutionary origins of the trait?
- 1.2 Can this information be used to develop molecular diagnostic kits which can reliably identify MHR populations in the field and rapidly distinguish these from TSR weeds for control purposes?
- 1.3 How many stress-response pathways are activated during the acquisition of MHR and what is their relationship to endogenous signalling responses?

The primary output of the work package will be the generation of diagnostic tools which will allow herbicide resistance diagnostics and auditing in the field and the study of the development of MHR to support our quantitative genetic studies.

Obj 1.1 – Genetic and biochemical fingerprinting of MHR in differing black-grass populations.

Work undertaken as part of a BBSRC-funded IPA project with Syngenta (completed September 2012) has identified profound alterations in the biochemistry and transcriptome of MHR black-grass relative to WTS populations. Based on pyrosequencing studies using the Roche 454

instrument, a total of 5985 black-grass genes have been assembled with one hundred being up-regulated more than 2-fold in the MHR Peldon population as confirmed by qPCR. As determined in *Lolium* (Fig. 1), a large number of the MHR unigenes were associated with signalling pathways, consistent with our hypothesis that there are potentially multiple regulatory routes leading to resistance. The 454 next generation sequencing studies in black-grass also identified a range of potential polymorphic molecular markers (SSRs and SNPs) which can be used to establish the provenance and genetic relatedness of the populations identified by the consortium. Using the recently acquired Ion Torrent facility within the Centre for Novel Agricultural Products (CNAP) and supported by dedicated support from the associated informatics group, these next generation sequencing studies in black-grass will be extended to include the new resistant populations collected in the audit conducted at Warwick. Full transcriptome analysis will be applied to populations which on the basis of herbicide testing studies at Warwick show an MHR phenotype, with qualitative and quantitative changes in expressed genes determined relative to genetically related WTS isolates. Based on current costings, this would enable the consortium to perform 3 replicated sequencing experiments for 10 MHR and 10 WTS black-grass populations to provide the extensive transcriptome coverage required for subsequent genotyping studies. As needed, quantification of specific transcripts of interest will be confirmed by qPCR. Based on these studies 20-30 genes will then be selected as variant markers for both resistance and population which can then be used for Amplicon sequencing for subsequent genotyping studies of individual plants and populations identified in the field in **WP2**, and resulting from the breeding experiments in **WP3**. In addition, for SNP and SSR analysis the group at York have access to a SNPLine and automated fragment analyzer facility to allow for high throughput genotyping analysis. Black-grass plants/populations will also be assessed for variations in their biochemical phenotypes using both targeted and non-targeted approaches. In the targeted assays, the plants will be assessed for changes in a) herbicide detoxification by measuring glycosyltransferase and glutathione transferase activities; b) stress metabolism as determined through flavonoid profiling using LC-MS and c) antioxidant protection as defined from their thiol content (glutathione, hydroxymethylglutathione) and related dependent enzymes (thioltransferase, dehydroascorbate reduction) using methods developed previously by the group¹⁵. In recent studies, we have also developed non-targeted metabolomics using Progenesis software to map changes in metabolism associated with MHR, using a combination of LC coupled to high resolution MS/MS approaches to identify additional compounds. These studies have revealed a further level of disruption in fatty acid oxylipin metabolism, which is potentially associated with the signalling underpinning MHR and can add further diagnostic discrimination to the underpinning mechanisms of resistance.

The intention of these studies is two-fold. Firstly to address the question as to how groups of genes and metabolites vary in the differing MHR populations. By mapping groups of perturbed (up- and down-regulated) genes and metabolites we will directly address the question as to whether MHR is essentially identical in independently evolved populations, differing only in the degree of modified expression, or whether it differs qualitatively, with different signalling pathways being differentially enhanced or suppressed. As a quantitative and polygenic trait, this approach will help unravel the signalling networks present that underpin MHR. Secondly, this work package will identify genes and metabolites which can then be used as reliable biomarkers for MHR for development into diagnostic tools in **Obj 1.2**.

Obj 1.2 – Developing molecular diagnostics to study MHR in black-grass

Classical tests for herbicide resistance in grass weeds currently require the collection of seeds, heat treatment to break dormancy, seedling propagation and spray trials with graminicides, acting on differing modes of action. For example the RISQ test developed by Syngenta has been widely adopted³⁰. Typically such tests, while highly definitive, take 6 weeks to perform and require labour-intensive transplantation of seedlings into glasshouses. With these limitations in mind, it is proposed to develop a lab-based diagnostics platform that can discriminate between the major types of resistance (MHR and TSR) in black-grass seedlings, which can then be refined for deployment in the field to inform weed control strategies within the growing season. The approach adopted relies on the formation of hydroperoxides and oxidation products as a consequence of the derailment in primary plant metabolism following herbicide treatment, irrespective of primary mode of action¹⁴. The resistance screens are based on the detection of oxidation products either directly (malondialdehyde), or through colourimetric assays based on hydroperoxide formation *in planta*

after infiltration with dichlorofluorescein diacetate (DCFDA) or 3,3-diaminobenzidine (DAB), or following simple extraction (luminol or xylenol orange). These methods are already used to determine disease resistance responses in plants³¹, with the xylenol assay already developed to measure herbicide injury in black-grass¹⁵. Initial studies will use whole leaves, with the intention of reducing the sample size of leaf segments so as to maximise the sampling from individual plants. For validation purposes, foliage from black-grass seedlings from authenticated TSR, MHR and WTS populations will be excised at the base and placed in solutions of graminicides acting on acetyl-coenzyme A carboxylase (ACCase = aryloxyphenoxypionate herbicides), acetolactate synthase (ALS = sulphonylureas) and photosynthesis (phenylureas) respectively. In primary screens plants will be analyzed using non-targeted metabolomics to both monitor the rate of herbicide metabolism and the appearance of endogenous oxidation products such as malondialdehyde (following on-line derivatisation) to confirm the biochemical detoxification and oxidative injury phenotypes over time. Having established optimal treatment parameters, the experiments will be repeated and the plants either extracted, or fed with peroxide-sensitive dyes and the resulting colour formation which related to oxidative damage recorded. Plants which show reduced injury to specific herbicides only will be classified as showing TSR, while seedlings showing reduced oxidation to several graminicides would be classed as exhibiting MHR. Following validation, the assays will then be worked up into different practical formats for primary field testing. In a further strand of practical resistance assay development, we will also develop immunodiagnosics based on the detection of *AmGSTF1* as a marker for MHR in black-grass^{15,17}. The diagnostics method proposed would use immobilized (and existing) antisera to *AmGSTF1* and the constitutively expressed tau class *AmGSTU1* arrayed in tandem on membranes inserted in lateral flow devices³². Black-grass leaf tissue is then subjected to a simple bead extraction and the homogenate applied to the lateral flow device, such that a positive immunoreaction with the anti-*AmGSTU1* confirms assay functionality and a positive reaction with *AmGSTF1* a likelihood of MHR. The purpose of these rapid deployable assays will be to help prioritise the subsequent work-flow, with positively identified samples of interest then being shipped to the lab for more detailed metabolomic and genomic analysis.

Samples testing positive for TSR or MHR will then be subjected to more detailed analysis. In the case of TSR for ACCase or ALS targets, the nature of the underpinning mutation can be rapidly identified using the PCR based method described for black-grass³. For the weeds suspected to be exhibiting MHR, individuals will be tested using the amplicon technology derived from **Obj 1.1**, using markers which give clear diagnostic signals for multiple resistance based on their relative induction and functional diversity. In addition to the molecular genetic screens, the black-grass plants of differing MHR backgrounds will also be analysed for altered protein and metabolite expression, thereby linking genotype with phenotype. For differential protein analysis, samples from MHR and WTS plants will be resolved on large format gels and differences in their proteomes identified using differential dye technology, prior to MS-MS sequencing in the Technology Facility at York. Finally, we will use LC-MS based metabolite profiling to identify low molecular weight biomarkers of MHR which have the potential to be developed into high throughput assays based on MALDI-ToF type platforms. Candidate metabolites identified to date are largely polyphenolic in origin, being related to flavone and anthocyanin secondary metabolites. This multiply tiered diagnostics approach will both generate useful tools to pre-emptively identify MHR/TSR in the field, as well as providing quantitative biochemical and genetic monitors for the evolution of resistance in **Obj 1.3**.

Obj 1.3 – Studying the molecular evolution of MHR

For each of the 540 seed families produced in **Obj 3.1**, leaf material will be taken from six individuals, two each of which will have been treated with an ALS and an ACCase herbicide, respectively, and two of which will not have been treated with a herbicide. Using the quantitative transcriptomic and metabolomic approaches developed in **Obj 1.1** and **Obj 1.2** we will assay these individuals for variation in gene expression and metabolite profiles. In the case of the *AmGSTF1* isogene family, emphasis will be placed on measuring the expression of individual members using specific probes based on 3'-UTRs, to determine if sequential activation is occurring. As an adjunct to the screens of MHR populations identified during the resistance audit (**Obj 2.2**), these studies will provide an important and insightful link between quantitative genetic and molecular genetic approaches. This integration between **WP1** and **WP3** will help to i) confirm that heritable

differences in MHR are associated with transcriptomic and metabolomic markers ii) indicate how variation in the level of phenotypic MHR is associated with genotypic variation iii) provide evidence for the importance of constitutive versus induced responses in MHR and finally, iv) indicate which genotypic changes are associated with pleiotropic impacts on plant fitness and associated life history traits.

We will also consider the potential for epigenetic mechanisms such as DNA methylation and covalent histone modifications on the development of MHR, as these modifications can have a major influence on gene expression and are responsive to changes in environment²¹. In particular, changes in DNA methylation status can be both mitotically and meiotically stable and therefore potentially provide a mechanism to allow stable propagation of the changes in gene expression which underpin MHR over multiple-generations. Similarly, histone modifications are dynamic and knowing which modifications are associated with a particular gene will be informative regarding the mechanism of activation or repression. The strategy to be adopted here will be to assess the potential for epigenetic mechanisms to contribute to the evolution of MHR, rather than to fully define the associated molecular mechanisms. This component of the work will be led by Dr Louise Jones, University of York, an expert on plant epigenetics. Genes that will be screened for potential epigenetic modifications will be selected for based on their induction by MHR, as defined from the studies in **Obj 1.1** and potential regulatory functions. An obvious target for such analyses will be the *amgstf1* gene promoters, which have already been identified in previous work. However as needed the promoters of additional genes of interest will be sequenced from genomic DNA. As a first level experiment, the potential for modified DNA methylation within promoters will be investigated using bisulfite sequencing, with chromatin immunoprecipitation (ChIP) used to assess changes in histone modifications using commercially available antibodies against both active (eg. H3K4me, H3K9ac) and repressive (eg. H3K9me, H3K27me) modifications. These studies will be performed on genes derived from individuals identified to have high levels of MHR in the quantitative genetic experiments (**Obj 3.1**).

In a parallel strand of activity, we will grow WTS black-grass under a variety of conditions imposing different abiotic stresses, including water stress, Nitrogen limitation, high temperatures and exposure to wounding. The Edwards lab have recently demonstrated that several key components of the MHR phenotype in black-grass can be replicated by treating WTS plants with herbicide safeners, which are chemicals that are routinely co-formulated with graminicides in wheat to increase herbicide selectivity¹⁵. In addition, we have demonstrated that *AmGSTF1* can be induced in WTS weeds exposed to water stress. Cumulatively, these studies suggest that the MHR phenotype can be replicated by applying selective natural stress treatments. By applying abiotic stress treatments singly and in combination, we will attempt to recreate the MHR phenotype as determined using the assembled diagnostic tools through an alternative route to herbicide selection. Dependent on the results of the epigenetics experiments we will look for evidence of similar regulation being exerted as a consequence of abiotic stress selection. This experiment will provide useful information in revealing the endogenous stress signalling pathways invoked in MHR as well as providing valuable insight into the interactions between the environment and the evolution of multiple resistance in the field.

Work Package 2. *Black-grass population monitoring and resistance audit.* (Rob Freckleton, University of Sheffield; Paul Neve, University of Warwick; and Rob Edwards, University of York).

This work-package will address the following:

- 2.1** Conduct surveys to provide the long-term data for parameterising and validating models of black-grass dynamics.
- 2.2** Perform a resistance audit across the range of black-grass distribution for key herbicide modes of action.

The primary output of the work package will be a baseline dataset on weed abundance, MHR and TSR status, and historical farm management

Obj 2.1 – Field monitoring and

Field monitoring method – background. Previously we have set up a monitoring system in which 10 fields in each of 47 farms have been surveyed. The farms were chosen to represent a range of

farming practices typical of lowland UK agriculture. Farmers were chosen by a combination of previous contacts (with those selected chosen to reflect a range of types, including contract managed, owned farms, and membership or not of professional and conservation schemes, such as TAG, ELS, LEAF) and random sampling (based on post-code in the Yellow Pages). The farms are distributed across 3 counties (Lincolnshire, Bedfordshire and Norfolk) in which arable agriculture predominates. Our sample was not statistically significantly different from the region-wide average in the Farm Business Survey.

An area of 2ha in each field has been monitored, with the monitoring being based on a grid system consisting of 20m x 20m quadrats. In total we monitored c. 98000 quadrats over an area of 900ha. Researchers walked each field assigning a weed density state to quadrats based on the density of weeds present. The weed density states were assigned on a 5 point scale (Zero, Low, Medium, High, Very High). This 5 point scale was determined based on the quartiles of density recorded in the Farm Scale Evaluation of GM crops³³. We conducted trials to validate this method of surveying and in preliminary work showed, using detailed density mapping of selected 20m x 20m quadrats, that the density states correlate very well with local densities, and that there is c.90% agreement between observers.

For the current project these data provide background information on the prevalence of weeds at a large scale, as well as information on management. Of the fields studied a large fraction (60%) were cropped with winter wheat, and of these a high proportion (71%) contained black-grass. We will use this information as the basis for identifying study farms with historically high densities of weeds. The data from 2007-2011 will be used in the modelling, and the study system used as the basis for setting up a survey for this project.

Population dynamic surveys. We will use the protocol we have developed to survey populations of black-grass. Concentrating on those farms with a high proportion of winter wheat (In our current modelling we are dealing with 29 farms in which the cropping is dominated by winter wheat), the existing plots will be relocated and a new set of surveys conducted. We will also locate a series of 20 additional sites for this project. The sites will be chosen to increase the geographic coverage of the project, and will include sites in Yorkshire and the Warwickshire. The new sites will be selected using the methodology used to select the original 47 sites.

Contribution to resistance audit. In order to provide a set of populations for analysis in the resistance audit in **WP2**, the data from 2007-2011, together with information from 2013 will allow us to identify 150 populations (i.e. fields) of black-grass that vary in average field densities. These fields will be located in fields that we have previously monitored. In doing so we will be able to identify fields that, based on previous records, have typically had low or high densities, as well as to stratify our sample in relation to management (rotation, frequency of herbicides), edaphic (especially soil), and socio-economic factors (e.g. farm size, owner versus managed farms).

Seed survey. In 2013, the survey will include systematic seed sampling, in addition to the seed collection undertaken for the resistance audit. We will subsample 20 x 20m plots at the level of a 1 x 1m to determine seed production as a baseline estimate of plant fitness as a function of density-state. In each field we will harvest whole plants from each of 10 20m x 20m quadrats in each density state (where possible). These samples will be separated into vegetative and seed biomass. The vegetative biomass will be dried in an oven at 70C for 72 hours, and weighed. The seed will be counted and stored. Germination trials will be used to determine germination rates and dormancy.

Management history. The history (going back for 8 years or as long as information is available, if less) of field and farm management will be documented from farm diaries. This information will be fed into modelling analyses (below). Particular attention will be paid to herbicides. We will record the amount and type of active ingredient sprayed on each field. The farms monitored to date have been mapped and the locations of major features that might be important for biodiversity have been recorded. These include the locations of hedgerows, ditches, roads, trees and farm buildings. The same will be done for the farms newly included in this project. The information from the management history will feed into the resistance audit (**WP2**) and the information on habitat features can be used in modelling habitat heterogeneity for wider impacts (**WP5**).

Analytical approaches. The weed population dynamic data will be analysed using statistical techniques recently developed by ourselves, using maximum likelihood and Bayesian procedures

to correct field scale estimates of density state frequency for misclassification. The corrected frequencies of each density state (i.e. discrete data) can be used to estimate the mean and variance of the density of weeds at the field scale (i.e. on a continuous scale³⁴). The data on weed abundance will feed into the resistance audit (**WP2**), allowing us to determine whether measured resistance is correlated with population densities, as well as correlating with management (below & **WP4**). The contribution of seed-banks to population growth can be estimated by isolating those quadrats which had zero density at time t (and no occupied neighbours) and calculating the proportion in each non-zero density state at time $t+1$. This measures directly the contribution to current densities of weeds of recruitment solely from the soil seed-bank in plots in which there were previously no weeds. We are currently developing more complex spatial analysis in order to estimate dispersal effects between neighbouring quadrats. The data will also be used in **WP4** for parameterising models, and for up-scaling from detailed local-scale models (see below).

Obj 2.2 – Resistance audit.

Phenotypic herbicide resistance assays. Over the last three years as part of other funded projects, the Neve lab has collected seed populations of black-grass from across the UK range of the species. In year one of the project, these 60-70 populations will be phenotyped for MHR as described below. During 2014, a standard seed sampling protocol will be employed to collect black-grass seed from a further subset of 150 populations monitored in **Obj 2.1**. Collected seed will be cleaned, dried and subjected to a standard dormancy breaking treatment (30-35°C dry storage for 4-6 weeks) prior to the commencement of herbicide bioassays under standard glasshouse growth conditions. The resistance status of all populations will be determined by dose response for two herbicide modes of action, frequently used for black-grass control (acetyl-coenzyme A carboxylase (ACCase) inhibitors and acetolactate synthase (ALS) inhibitors). Dose response assays will be conducted according to well-established protocols. Herbicides will be applied to pot-grown plants at appropriate growth stages using an experimental pesticide track sprayer. Plant mortality and biomass of survivors will be assessed 4 weeks after herbicide application. Standard non-linear regression techniques for dose response analysis will be conducted to establish LD₅₀ and GR₅₀ parameters. The frequency and extent of herbicide resistance in surveyed populations will be considered in relation to data collected in **WP3** on the density, dynamics and management use histories of individual populations. Variation in sensitivity of populations to two doses of up to four additional herbicide modes of action will also be assayed under the conditions described above. Where resistance to these modes of action is evident, and as resources permit, more detailed dose response assays will be performed to quantify resistance at the population level.

Determination of genotypic/mechanistic basis of resistance. All phenotyped seed populations will be subject to SNP/SSR DNA analysis to establish the genetic provenance of populations included in the resistance audit. In black-grass populations where resistance is detected above a certain threshold level, leaf samples will be taken from approximately 20 surviving plants. PCR-based and biochemical assays will be performed to identify the presence and frequency of known mutations for ACCase and ALS target-site resistance, as well as the presence of metabolism-based MHR determine the mechanisms of resistance within populations. In addition to identifying the relative extent of the different mechanisms, their co-occurrence and frequency within populations, these genotypic assays will enable us to clearly relate phenotype to genotype. In particular, it will be possible to relate the level of resistance (determined by dose response analyses) to underlying mechanisms to provide an indication of the relative fitness advantage of different mechanisms, alone and in combination. Knowledge of the mechanisms present in individual populations will also provide a means to determine the fitness costs associated with resistance mechanisms, singly and in combination. Data relating genotype to phenotype in the presence and absence of herbicides will provide important inputs and insight for modelling in **WP4**.

Work package 3. Genetic architecture and inheritance of MHR. (Paul Neve, University of Warwick; Dylan Childs & Rob Freckleton, University of Sheffield; Jarrod Hadfield, University of Oxford; Rob Edwards, University of York)

This work-package will address the following:

- 3.1 Determine the quantitative genetic architecture of metabolic herbicide resistance in selected UK black-grass populations

3.2 To explore trade-offs between defence (MHR/stress tolerance), growth and reproduction in black-grass populations.

The primary output of the work package will be to obtain the relevant genetic parameters that determine the rate at which resistance will evolve, and to determine whether genetic variants confer general resistance to herbicides or resistance to specific herbicides.

Obj 3.1 – Quantitative genetics of MHR in black-grass.

For the first time, evolutionary quantitative genetic approaches will be employed to determine the heritability of MHR in black-grass as well as the genetic correlations between MHR and life-history traits in order to measure the costs associated with MHR. Taking into account practical considerations and the outcrossing nature of the species, preliminary power analyses have identified an optimal crossing design whereby each of 180 'male' plants will act as a pollen donor to each of 3 'female' plants, to provide 540 seed families. Comparing the resulting full-sib and paternal half-sib families will allow us to estimate the amount of additive genetic variance segregating for MHR whilst controlling for possible maternal effects and non-additive genetic effects that may not contribute to an evolutionary response. Plants from six populations identified to have a range of MHR levels in **Obj 2.2** will be used to produce these crosses in years one to three of the project. The parents of each seed family will come from the same population. Plants will be grown to reproductive maturity under standard glasshouse conditions and crosses will be performed at the appropriate time. After crossing, 'female' plants will be isolated in clear, pollen-proof bags to prevent any subsequent cross-pollination between plants in the glasshouse. Plants will be grown to maturity and seed will be collected.

For each of four herbicides commonly used for black-grass control in the UK, five individuals from each of the 540 seed families will be exposed to one of three herbicides doses. These doses will include the field application rate and two sub-lethal doses. Plants will be grown and maintained in the glasshouse under standard, replicable growth conditions. Herbicides will be applied with a pesticide track sprayer. Mortality of herbicide-treated plants will be assessed four weeks after spraying. This ambitious design is achievable as each of the four herbicide assays can be completed in series within an eight week period under highly standardised glasshouse conditions. The results from this breeding design will provide us with precise estimates of the genetic variation that underpins herbicide resistance, and will allow us to link this variation to molecular variation identified in **WP1** (see **Obj 1.3**).

Obj 3.2 – Trade-offs with resistance in black-grass.

Surviving plants from **Obj 3.1** will be destructively harvested after four weeks and biomass recorded. In addition, an additional five plants from each seed family will be grown to maturity under identical glasshouse conditions but in the absence of herbicides. A range of non-destructive measurements of plant size (tiller number, height) and phenology (flowering time, seed maturity) will be made at appropriate times during the life cycle. At reproductive maturity, fecundity of individuals will be determined. Finally, seed viability and mean seed dormancy in each family will be estimated in separate seed germination assays. Combined with **Obj 3.1** we will be able to estimate the genetic correlations between resistance and life history traits and provide unprecedented insight into the evolutionary trade-offs that exist in the evolution of herbicide resistance. The nested paternal half-sib/full-sib experimental design is widely used and standard statistical genetic techniques can be used to estimate the relevant parameters. Slight complications arise when the phenotype is not continuous (for example, survival) particularly in multi-trait models. However, threshold models can be used to address this issue, and software has been developed by ourselves to accommodate these complexities³⁵. Parameters from these analyses will provide inputs for the eco-evolutionary models produced in **WP4** and will allow us to determine how fast MHR will evolve and whether this rate would be reduced or accelerated by using herbicide rotations, sequences and/or mixtures.

Work package 4. *Eco-evolutionary modelling of black-grass populations.* (Dylan Childs and Rob Freckleton, University of Sheffield; Paul Neve, University of Warwick; Jarrod Hadfield, University of Oxford).

This work-package will address the following:

- 4.1 Develop a novel integrated modelling framework to explore the joint eco-evolutionary dynamics of weed populations in agri-ecosystems.
- 4.2 Predict the management implications of interactions between ecological and evolutionary processes.
- 4.3 Evaluate the robustness of these predictions to assumptions about spatial processes, stochasticity and genetic architecture.

The primary output of the work package will be a data-driven assessment of the evolutionary potential of black-grass populations for MHR evolution, enabling the wider implications of management to be addressed.

Obj. 4.1 Developing a new eco-evolutionary model of black-grass population dynamics

Though our models will be parameterised for black-grass, we aim to build a framework that is general enough to be adapted to other systems.

Baseline Population Model. A general model describing the density and size-/age-structured dynamics of local weed populations will underpin our analyses. This will couple two sub-models (Fig. 5). The first will be a discrete time model capturing the among-year dynamics of the seed bank, derived from classic ecological models of annual weeds³⁶. Our model will incorporate explicit age-structure in order to track among cohort variation in the mean phenotype due to selection (see *Evolutionary Analysis*). This will allow us to evaluate the impact of historic patterns of selection on the evolutionary potential of the seed bank. The second sub-model will be based on the integral projection model (IPM³⁷⁻³⁹), which describes the within-season dynamics of newly established weeds in terms of individual-level processes (i.e. recruitment, weekly growth and survival over the growing-season, and end-of-season reproduction). Classic weed population models typically subsume these processes into a single “fecundity function”; such an approach is insufficient for our purposes. An explicit within season model will allow us to: 1) understand the impact of trade-offs between growth and MHR; 2) track multiple cohorts that germinate in different weeks; 3) make predictions about the abundance and size-structure of the weed population, enabling us to incorporate realistic crop yield-loss functions; 4) explore the impact of varying the timing of herbicide application; 5) compare our model predictions (e.g. population density and size structure in different settings) to the data collected in **WP2**. Moreover, adopting an IPM framework for the within-season sub-model offers the best prospect of meaningfully tying our model to the data collected in **WP3** (see *Parameterisation*).

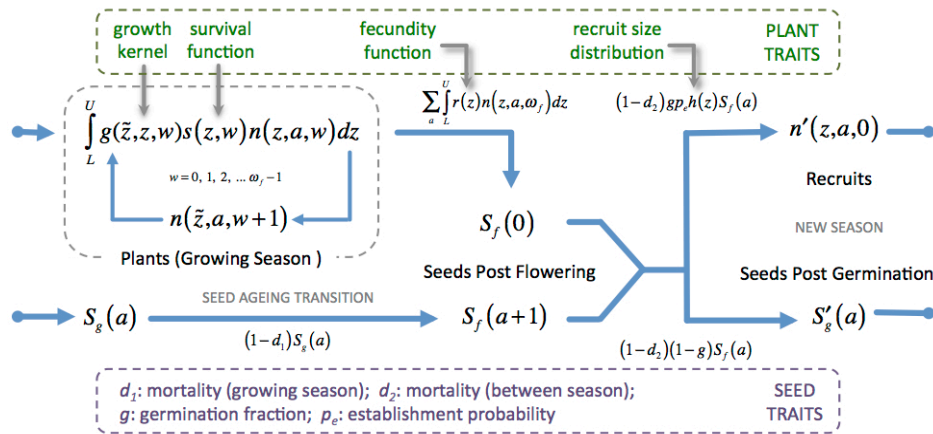


Fig. 5. Putative weed population model applicable to black-grass constructed from a pair of coupled sub-models: (1) an age-structured between-season model of the seed bank; (2) a size-/age-structured within-season model of established plants. The within-season model tracks the population density and size structure of black-grass, and can be (partially) parameterised from the data collected in **WP2**.

Evolutionary Analysis. The baseline population model will be used to evaluate the strength of selection operating on MHR and life history traits, via calculations of the sensitivity of population growth rate to changes in the model parameters. Parameter sensitivities are a widely used metric for summarising selection in demographically structured populations⁴⁰. However, we aim to predict the expected short- and long-term responses to selection on MHR and life history traits, allowing for genetic constraints embodied in the associated G matrix characterised in **WP3**. Since there is no exact analytical approach available for predicting multivariate trait evolution on both short and long time scales in age-structured populations, we will use brute force simulation to derive these predictions. This takes advantage of the fact that selection only acts on certain traits at each stage in the life cycle: starting with a newly emerged cohort of recruits, selection acts first (jointly) on plant survival/resistance and growth, then on plant fecundity, and finally on seed dormancy and

survival. During each episode of selection we will directly evaluate the change in the population mean breeding value (the genetic selection differential, S_G) by extending the baseline model to include the distribution of breeding values associated with the corresponding traits. Once S_G has been evaluated, the correlated response to selection in unselected traits can be calculated, and the updated model parameters applied to evaluate response to selection at the next life stage. Each seed cohort will therefore have a unique set of traits (model parameters) associated with it that reflects the historical management regime and ecological context experienced.

Parameterisation. A key strength of the IPM is that it can be directly parameterised from regression models describing the state-dependent vital rates of individual plants. Such models underpin the analysis of the breeding design in **WP3**. For example, a simple model for plant growth might take the form $\tilde{z}_i = \alpha_0 + u_i + \alpha_z z_i + \varepsilon$, where \tilde{z}_i is the current (log) size of individual i , z_i is their size next week, u_i is the individual's breeding value, and the parameters are the fixed intercept and slope of the growth model. Plants experience resource competition in the field, and their growth and survival trajectories respond to these pressures. To ensure that the modelled growth, survival and fecundity functions are relevant to the field, we will aggregate new observational data (**WP2**), pre-existing experimental data (PN, unpublished), and review the published literature to explore a realistic range of the parameters. Crucially, we do not expect the relative performance of plants with different breeding values to differ qualitatively among greenhouse and field conditions; e.g. a genotype that is competitively inferior (but resistant) in the greenhouse is unlikely to be competitively dominant in the field in the absence of herbicide application. By ensuring that our IPM is parameterised with models of the same form as those applied in **WP3**, we will ensure that the estimated genetic correlations remain directly informative to the modelling. Finally, we will use estimates of germination rates, annual dormancy and seed production derived from **WP3** to parameterise the seed bank component of our model.

Canonical Management Scenarios. Predictions from the model will be derived under a range of herbicide-dominated versus integrated weed management scenarios. 'Business-as-usual' scenarios will explore these dynamics in wheat-dominated crop rotations with little or no cultural weed control where herbicide control is dominated by a single mode of action. Models will be used to explore the effectiveness of herbicide mode of action rotations, sequences and mixtures for attenuating the rate of resistance evolution. Additionally, we will assess the potential impacts on the probability and dynamics of resistance evolution of novel crop rotations, which are less dominated by wheat and which include spring-sown crops and fallowing. As well as exploring impacts of herbicide and crop diversity, models will examine the benefits of cultural weed management, which will for example, aim to reduce black-grass recruitment and fecundity through soil seed bank management and enhancement of crop competitiveness. Other novel methods for reducing replenishment of soil seed banks will also be explored.

Obj 4.2 Exploring the management implications of interactions between ecological and evolutionary processes.

The baseline population model will first be used to understand how black-grass life history and ecology interact with management to determine weed abundance and associated changes in crop yield at the field scale, in the absence of evolution. That is, we will undertake an analysis of the ecological dynamics of black-grass that mirrors previous studies of weeds to benchmark the performance of our model against established approaches. With a well-characterised model in hand we will then examine the coupled eco-evolutionary dynamics of black-grass to address our core questions. In each case, we will examine the implications of adopting the canonical management scenarios outlined in **Obj 4.1**. We will also revisit these predictions in the light of the future change scenarios identified in **WP5**. Key questions to be resolved include:

4.2.1 How fast will MHR evolve under different management scenarios? We will quantify the expected rate of MHR evolution (the change in population mean LD_{50} per year) under different scenarios, assuming a range of initial conditions. Changes in MHR and population density (or biomass) under herbicide selection are expected to be correlated. Therefore, we will also establish whether practises that minimise the rate of MHR evolution in the long term are also optimal for minimising black-grass density in the short term.

4.2.2 Does the evolution of MHR significantly increase the risk of TSR evolution? The evolution of MHR increases black-grass population density, potentially altering herbicide application rates,

which in turn impacts selection on resistance. Since the evolution of TSR will be influenced by these changes, we will examine the extent to which MHR facilitates the evolution of TSR (the mean and variance in time to emergence). If MHR is associated with significant costs, then it is likely that the evolutionary dynamics of MHR under herbicide selection will differ post TSR emergence. Therefore, we will also explore the coupled dynamics of MHR and TSR.

4.2.3 What role does genetic correlation among life history and resistance play in the evolution of MHR? The response to selection on suite of traits is a consequence of the strength and direction of selection on those traits, and their genetic (co)variances. We will investigate the extent to which genetic trade-offs among black grass life history and MHR (e.g. growth vs. survival under herbicide selection) constrain the rate of MHR evolution. We will also explore how genetic correlations among different herbicide modes of action might influence the performance of management practises.

4.2.4 What is the evolutionary potential for MHR in black-grass? It is well established that seed dormancy is a key adaption that buffers weeds against life in an unpredictable environment⁴¹. What is less well understood is the extent to which the seed bank can act as reservoir of genetic variation for a trait such as MHR. We aim to identify management practices that minimise not only the mean, but also the genetic variance in MHR in the seed bank. We will also examine the coupled evolutionary dynamics of MHR and seed dormancy.

Obj 4.3 An individual-based model (IBM) of black-grass: space and stochasticity.

In order to retain analytical tractability our baseline model will assume that black-grass populations are 'large' and that interactions occur at the field scale. We will construct a more realistic, though much less computationally efficient, individual-based analogue of our model to evaluate the robustness of the key predictions derived in **Obj 4.2**. Specifically, we will explore the implications of spatial processes (local density dependence and mating), stochasticity (demographic stochasticity and genetic drift) and genetic architecture (e.g. oligo- vs. polygenic inheritance). These models will also provide an important link between **WP4** and **WP2**, since the spatially resolved individual-based model outputs can be directly compared to the data collected in **WP2**. Specifically, we aim to compare the predicted relationships between weed density, resistance and management practises to the observed patterns, both within and among fields. Development of an IBM will also enable us to compare the results of our work to similar published models^{e.g. 42}.

Work package 5. Wider impacts. (Ken Norris, University of Reading; Rob Freckleton and Dylan Childs, University of Sheffield).

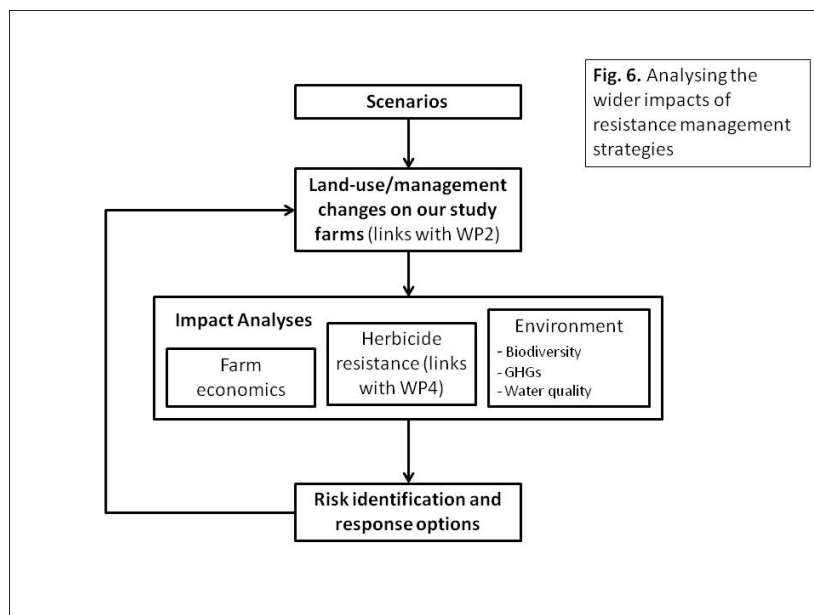
This work-package will address the following:

- 5.1 Develop future scenarios of land-use change and management, including potential strategies to minimize or reduce herbicide resistance.
- 5.2 Translate these scenarios into the land-use and management changes we would expect to see in the countryside.
- 5.3 Assess the potential impacts of these scenarios on herbicide resistance, farm economics and the environment.
- 5.4 Identify significant risks in terms of herbicide resistance, farm economics or the environment associated with each scenario, design management responses and re-assess risks.

The primary output of the work package will be potential solutions to resistance management that also minimize adverse economic or environmental impacts.

To achieve this, we plan to explore the economic consequences of resistance management strategies using farm-scale cost-benefit analysis. Environmental impacts of resistance management strategies will be analysed using approaches that enable us to consider farmland biodiversity, water quality and greenhouse gas emissions. These analyses will draw on farm management data collected in **WP2**; and apply the eco-evolutionary models developed in **WP4**. All of these datasets will then be used to identify key trade-offs between resistance management strategies, economics and the environment and to identify additional measures that may be required to address significant trade-offs. In this way, we will seek solutions to resistance management that also minimize adverse economic or environmental impacts. This approach treats

herbicide resistance as an adaptive management problem. The steps we plan to follow in this process are illustrated in Fig. 6.



Initially we plan to develop a series of scenarios or storylines that describe how land-use and management may change in the future (i.e. over the next 20-30 years). This is important because the management of herbicide resistance cannot be considered in isolation from the range of other drivers, such as markets, policy, technology and the environment, that interact to shape UK agriculture. To develop a series of scenarios for our project, we plan to draw on previous work that has developed land-use change scenarios for the UK, including the *Foresight Land-use Futures* Project and the

UK National Ecosystems Assessment (NEA). We will supplement these scenarios with the development of some new ones of our own that address specifically how herbicide resistance might be managed. In this way, we will aim to produce a small number (6-8) of scenarios covering a range of plausible land-use futures (e.g. maximizing agricultural production, greening the countryside, reducing herbicide resistance, business-as-usual, etc) that we can then explore in more detail. We plan to involve our *Stakeholder Group* directly in this scenario development work (see our *Impact Plan* for details) to ensure that the analyses we undertake in **WP5** are as realistic as possible, and well aligned with the needs and interests of the main stakeholder groups associated with our project.

Next, we plan to translate these scenarios into the land-use and management changes we would expect to see in the countryside. To do this, we plan to use the study farms and associated land-use/management data collected in **WP2**. For each of our scenarios, we will produce new land-use and management plans for each of our study farms. We will involve the relevant farmers in this process. In this way, we plan to describe how land-use and management on our study farms is likely to change under each scenario. These data will then form the basis of our impact analyses that explore the consequences for farm economics, herbicide resistance and a range of environmental endpoints (Fig. 6).

We will use existing farm management information from our study farms (collected in **WP2**) to calculate the costs and benefits of existing conventional management based on herbicides, and combine these data with resistance survey data to explore the relationships between the costs and benefits of weed control, and resistance at field and farm-scales. In this way, we will develop a baseline economic analysis of contemporary management on our study farms. We will then use the scenarios developed above to explore more generally how economic costs and benefits are likely to change as farm management changes. Appropriate costs and benefits will be derived from existing management information from our study farms, the literature and large-scale datasets such as the farm business survey^{43,44}.

We plan to explore the consequences of our scenarios for herbicide resistance by applying the eco-evolutionary models developed in **WP4** (details above).

We plan to explore three environmental endpoints – biodiversity, greenhouse gas emissions (GHGs) and water quality (Fig. 6). These endpoints have been selected because there is clear evidence that intensive agriculture can have a detrimental effect on these components of the environment^{45,46}, and there are a range of current policy initiatives aimed at reducing the impact of agriculture on these endpoints (e.g. agri-environmental management, carbon accounting, water framework directive). Exploring multiple endpoints is also important because trade-offs involving

farm economics or herbicide resistance might vary between environmental endpoints. We plan to explore the implications of our scenarios for biodiversity, GHGs and water quality using published approaches and existing datasets.

We plan to assess farm-scale biodiversity impacts in two main ways. First, we have previously developed an approach to assessing the risks of agricultural change to over 300 UK plants and animal species, including arable plants, bees, butterflies, birds and mammals^{45,47}. This approach simply assesses the extent to which a change in land-use or management detrimentally affects the ecological requirements of each species. We can, therefore, use the approach to identify the species most at risk from land-use and management changes associated with our scenarios by comparison with contemporary land-use and management. We can also use the approach to identify the ecological mechanisms associated with these risks. Second, we can use a combination of statistical and mechanistic models to explore how farmland bird populations are likely to respond to the land-use and management changes associated with our scenarios^{48,49}. Farmland bird population trends are an important indicator of the impact of UK farming on biodiversity, so quantifying whether our scenarios suggest improving or deteriorating trends for farmland birds is important.

We plan to assess the implications of our scenarios for GHGs using the *Cool Farm Tool* (CFT) (www.coolfarmtool.org). CFT is a farm-scale GHG calculator designed to enable farmers to measure and understand the sources of on-farm GHG emissions. We plan to use CFT to estimate changes in GHGs associated with our scenarios in comparison with contemporary farm management conditions. We can also use CFT to identify the mechanisms through which GHGs are likely to change. We plan to use published simulation approaches to assess the water quality implications of our scenarios by focusing on nitrogen (N) and phosphorus (P) turnover and runoff^{46,50,51}. We will review the applicability of these simulation approaches to our study farms. We will then apply these models to estimate farm-scale changes in N and P associated with our scenarios in comparison with contemporary farm management conditions.

Our impact analyses will enable us to identify risks in terms of farm economics, herbicide resistance and the environment associated with specific farms, farm types, particular scenarios or a combination of these (Fig. 6). Having identified particular risks, we then plan to consider what land-use or management response options might, in principle, be deployed to reduce these risks. Again, we plan to involve our *Stakeholder Group* in this process. We will then repeat our impact analyses in order to estimate the changes in farm economics, herbicide resistance and environmental endpoints we might expect from the response options to quantify the extent to which risks have been reduced or eliminated, and to see whether new risks have emerged. This iterative process can be repeated until acceptable levels of risk have been reached, or until fundamental trade-offs have been identified such that reducing risk in one dimension inevitably increases risk in another and *vice versa*.

Strategic value of the programme to the BBSRC as delivered through a LOLA

As recognized explicitly in BBSRC's strategic plan, the security of food production and 'sustainable intensification' are among the council's most important current drivers in both science and policy. To date, much research emphasis has been placed on the development of crop based genetic technologies to achieve yield goals. However, the falling off of increased cereal productivity per Hectare since the mid 90s, despite anticipated enhanced yields based on genetic improvement, are now identifying a new focus for food security research, notably around agronomy and crop protection. With respect to crop protection, the rapid development of resistance in agricultural pests, diseases and weeds is now a major concern, yet the respective funding portfolio for related publically-funded research has been in consistent decline for over a decade. In particular weed research activity in the UK is now at particularly low ebb, despite the scale of the threat to cereal productivity due to herbicide resistance in competing grass weeds. As such, a major strategic driver for this proposal is the desire to re-energize research in weed research, moving the subject on from being descriptive, to a more quantitative and solutions based science. Importantly, in delivering new solutions in combating herbicide resistance, the project considers the potential impacts of the proposed intervention measures from the outset, being closely focused on the needs of end users. As such, this proposal represents a unique 'agri-systems' approach in

generating and integrating basic knowledge relating to molecular mechanisms of resistance through to consideration of the ecosystem level consequences of herbicide resistance and novel weed management strategies. This central tenet of the programme is closely aligned to the BBSRC's strategy of "*Integration of the latest bioscience and modelling techniques is encouraged at all scales from molecules and cells to agricultural systems and landscapes*".

With respect to the value of the project being delivered through a Lola, a series of smaller projects would be unable to link the various elements in a manner required for developing large-scale and long-term predictions. A short-term experimental study over three years would provide new insights and data on resistance, but modelling and exploration of wider impacts are needed to fulfill the BBSRC's aim's expressed above in terms of an integrative approach. The evolution of herbicide resistance in weeds presents an excellent model in which to begin to expand the context of 'systems biology' so that a detailed knowledge of the molecular and cellular mechanisms of MHR can be considered at higher levels of organisation (individual, population, ecosystem) to address a major threat to sustainable crop production in the UK, and ultimately globally. Specifically the Lola will allow:

- (1) The experimental work to benefit immediately from the large-scale survey data, including using this to identify resistant populations, and in relating resistance to long-term management.
- (2) The work on generating diagnostic tools to be immediately tested and used in the large-scale surveys, allowing the benefits of BBSRC research to be immediately accessible to UK agriculture.
- (3) Linking experimental work on selection and trade-offs with demographic models will enable long-terms consequences to be understood.
- (4) The exploration of the wider-scale impacts will allow realistic management options to be prioritized and the likely conflicts with other environmental objectives immediately highlighted.

As well as potentially optimal strategies under different scenarios. A further aspect of the added value of the multidisciplinary approach proposed is the effect it will have on community building and raising the profile of Weed Science as a progressive component of integrated crop protection in the UK. Activity in this field of research has been in consistent decline in the UK in the public sector over the last 20 years due to a combination of changes in Departmental priorities and funding for herbicide research and the re-alignment of the agrochemical sector. As such, at a time when herbicide resistance in grass weeds have reached epidemic proportions the response from the academic sector to provide novel solutions has been fragmented and lacking in co-ordination. By using a combination of the latest molecular methods and sophisticated modelling based on systems type approaches the project will elevate herbicide resistance research in the UK to being at the forefront of the field internationally.

Strategic value of the application to partner institutions

This proposal is closely allied to key strategic priorities of the collaborating institutes. The **Centre for Novel Agricultural Products** at York has invested heavily in genetics technologies for fast-track breeding and through its close ties with the **Food and Environment Research Agency**, is now engaged in developing combined solutions for developing new crops which include integrated pest management and weed control. The **University of Sheffield** has placed food security at the centre of its flagship project Shine, which is a university-wide synthesis of work in food production and environmental protection. At Sheffield the **Department of Animal & Plant Sciences** is home to one of the largest groups of evolutionary biologists and plant scientists in Europe. The **University of Warwick** has established Food Security as one of its Global Priority Programmes. 'Food security' and 'environment' are two of the four major research themes in the newly formed **School of Life Sciences** at Warwick. These themes are integrally linked to the **Warwick Systems Biology Centre**, which represents an £11m investment by the University in multidisciplinary experimental and mathematical research. The **University of Reading** recognizes Food Security as a research area of major strategic importance and has created the **Centre for Food Security** to bring together researchers across campus to address the links between food, farming and the environment. This recent activity builds on past investments, such as the creation of the **Centre for Agri- Environmental Research** (CAER) over a decade ago, which together provide the University with significant research capacity in agri-environmental systems. More specifically, CAER has played a leading role in recent years in developing approaches to

assessing the biodiversity risks of agricultural change. The **University of Oxford** engages widely with many aspects of food security with research brought together under The Oxford Martin Programme on the Future of Food, an interdisciplinary programme of research and policy engagement concerning all aspects of the food system.

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