NEW TECHNOLOGIES TO COMBAT HERBICIDE RESISTANCE

Rob Edwards*, Agriculture Building, School of Natural and Environmental Sciences, Newcastle University, Newcastle NE1 7RU. Resistance to herbicides is becoming an increasingly serious problem in weed control. New approaches to overcoming this are outlined.

Herbicide resistance in problem weeds is now a major threat to global food production, being particularly widespread in wild grasses affecting cereal crops (Yu & Powles 2014). In the UK, black-grass (Alopecurus myosuroides) holds the title of number one agronomic problem in winter wheat, with the loss of production associated with herbicide resistance now estimated to cost the farming sector at least £0.5 billion p.a. (Varah et al., 2019). Black-grass presents us with many of the characteristic traits of a problem weed; being highly competitive, genetically diverse and obligately out-crossing, with a growth habit that matches winter wheat (Figure 1). With the UK’s limited arable crop rotations and the reliance on the repeated use of a very limited range of selective herbicides we have been continuously performing a classic Darwinian selection for resistance traits in weeds that possess great genetic diversity and plasticity in their growth habits. The result has been inevitable; the steady rise of herbicide resistance across the UK, which now affects over 2.1 million hectares of some of our best arable land (Hicks et al., 2018). Once the resistance genie is out of the bottle, it has proven difficult to prevent its establishment and spread. With the selective herbicide option being no longer effective, the options are to revert to cultural control; changing rotations and cover crops, manual roguing of weeds, deep ploughing and chemical mulching with total herbicides such as glyphosate. While new precision weeding technologies are being developed (Korres et al., 2013), their cost and scalability in arable farming remains unproven. As an agricultural scientist who has spent a working lifetime researching selective weed control, we seem to be giving up on a technology that has been a foundation stone of the green revolution. For me it begs the question, are we really unable to use modern chemical and biological technology to counter resistance?

I would argue the answer to that question is most patently no; solutions are around the corner if we choose to develop them. When bacteria or animals become resistant to multimillion dollar drugs, we do not abandon the therapy. Rather scientists establish the resistance mechanisms and develop chemical or biological treatments that suppress them. For example, the development of resistance to penicillin and related chemistries prompted the development of Augmentin, a drug that inhibited the lactamase enzyme in pathogenic bacteria that inactivated this class of antibiotics (reviewed by Gatadi et al., 2019). Similarly, in human cancer therapy, the development in multiple drug resistance in tumours has led to the development of drugs that target and inactivate the tolerance mechanism (Ruzza et al., 2009). In both cases, the key to these new interventions was to use the very best science to understand the molecular basis of drug resistance, which chemists could then target for selective inhibition. For the first time weed scientists are now gaining similar insights into herbicide resistance, opening up new possibilities to target this damaging trait.

To disrupt herbicide resistance we first need to understand it. Weeds evolve tolerance to herbicides through two overarching mechanisms (Yu & Powles 2014), termed target site resistance (TSR) and non-target site resistance (NTSR). In TSR, mutations in the genes encoding proteins targeted by herbicides result in the synthesis of enzymes, or structural polypeptides that show reduced sensitivity to chemical inhibition. Providing the protein is still functional in the plant, TSR provides a rapid and powerful evolutionary route to resistance to the specific class of

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herbicide chemistries having that mode of action. Once diagnosed, TSR can be counteracted by rotating herbicide modes of action to reduce the incidence of the mutation in the weed gene pool (Yu & Powles 2014). In addition, TSR-mutations often result in a fitness penalty to the weed, such that its incidence is also rapidly reduced on removal of selection by the respective herbicide. NTSR in contrast is a more complex beast, behaving as a multigene encoded trait that can vary quantitively to deliver anything from mild to severe cases of resistance. The molecular basis of NTSR is by its nature multifactorial and known to be closely linked to the enhanced metabolism and detoxification of herbicides, irrespective of their chemistry or mode of action (Yu & Powles 2014). Plants contain a diverse range of detoxifying enzymes we have termed the xenome, which through sequential phases of metabolism are able to biotransform an enormous diversity of herbicide chemistries (Figure 2). As such NTSR is very damaging when it evolves in weed populations, as it can result in a loss of control to multiple herbicides, including potentially chemistries yet to be developed. It is also clear that neither type of resistance evolves in isolation, with many black-grass populations evolving both TSR and NTSR (Hull et al., 2018). While there were many 100s of differences in gene expression between different MHR and herbicide susceptible weed populations, when we examined changes in abundance of soluble polypeptides we found a much simpler and characteristic alteration in the signature of proteins present. On identifying the MHR associated proteins, we were surprised to find that they showed greater similarity to the changes associated with MDR in humans, than to any known plant stress response. In particular, one black-grass protein, a glutathione transferase (GST), we termed AmGSTF1 was consistently associated with MHR, with its expression levels positively correlating with the levels of herbicide resistance observed. This protein was already known to us through an earlier research programme (Cummins et al., 2013), though this was the first time we could unambiguously link the expression of AmGSTF1 to multiple populations of black-grass that had independently evolved the MHR trait. The function of AmGSTF1 was rather puzzling to us, as it had very little ability to detoxify herbicides as might be anticipated from the central role of these enzymes in the plant xenome (Figure 2). On transforming other plants with the gene encoding AmGSTF1 we discovered that the transgenic recipient plants acquired tolerance to herbicides used against black-grass and underwent subtle changes in their biochemistry that we had previously observed to be associated with MHR in grass weeds (Cummins et al., 2013). Excitingly, for the first time we had isolated a gene from a wild grass that had a regulatory function in controlling the MHR trait.

This breakthrough immediately posed the question as to how GSTs could regulate MHR and our attention was immediately drawn to a case of apparent parallel evolution in plants and animals. Drug resistance in human tumour cells also involves a specific glutathione transferase, HsGSTP1 encoded by a gene that has evolved completely independently from AmGSTF1. In common with the black-grass enzyme, HsGSTP1 exerts its protective role through a combination of direct detoxification and complex regulatory functions (Ruzza et al., 2009). As HsGSTP1 is problematic in delivering effective chemotherapy in cancer treatment, it has been considered as an important drug target (Ruzza et al., 2009). One of the selective inhibitors developed that disables HsGSTP1 and counteracts MDR was based on the relatively simple pharmacophore 4-chloro-7-nitrobenzoxadiazole (NBD-Cl), that modifies the target protein chemically and disrupts its catalytic and signalling functions. On testing NBD-Cl on our AmGSTF1 protein, we were able to show it too modified the protein, reduced its enzymic activity and most excitingly reversed the MHR phenotype toward some selective herbicides in black-grass (Figure 3). As a proof concept, these studies showed that by targeting a protein involved in regulating MHR with chemical inhibitors, it was indeed possible to
reverse resistance and restore control using existing herbicides (Cummins et al., 2013).

The concept of herbicide synergists, compounds such as malathion and piperyl alcohol butoxide that reduce resistance to specific chemistries by inhibiting detoxifying xenome enzymes such as cytochromes P450 is well established in insecticide use, though largely restricted in use to being just a research tool when used with herbicides (Yan et al., 2019). Instead our recent findings open up the potential to develop a new generation of agrochemicals that act to disrupt the very mechanisms regulating MHR, a concept we term ‘MHR-busting’. Here we can learn from health care, where counteracting MDR mechanisms is a well established strategy in maintaining the efficacy of precious antibiotics and chemotherapeutic agents we can ill afford to lose. Conceptually the use of agrochemicals to regulate the activity of selective herbicides is already well established through the widespread use of safeners, that as co-formulants reduce crop injury by enhancing detoxification through a coordinated induction of xenome enzymes and transporters (Figure 2). Just like the MHR-busting NBD-Cl, safeners target the pathways that regulate herbicide resistance mechanisms and in terms of adoption are now an essential component technology in modern selective weed management.

To develop herbicide resistance-busting to the next level now needs us to develop AmGSTF1 inhibitors with more benign chemistries than NBD-Cl that can be developed into commercial products that satisfy environmental and safety regulatory requirements. In addition, we know that AmGSTF1 is not the only regulator of NTSR, so we now need to examine the other proteins linked to MHR in wild grasses. Importantly, the recent work showing the suppression of glyphosate resistance in weeds using RNAi applications to suppress the expression of herbicide resistance genes (Korres et al., 2019), demonstrates that we no longer just have to think about chemical inhibitors, we can also call upon the latest gene silencing technologies to disable the regulators of MHR. Whatever the technology used, actively counteracting MHR should now be a major priority in future herbicide technology programmes both to safeguard the chemistries we have remaining to us as well as extending the life of active ingredients yet to be commercialised.

References


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