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Expanding the eco-evolutionary context of herbicide resistance research

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Abstract

The potential for human-driven evolution in economically and environmentally important organisms in medicine, agriculture and conservation management is now widely recognised. The evolution of herbicide resistance in weeds is a classic example of rapid adaptation in the face of human-mediated selection. Management strategies that aim to slow or prevent the evolution of herbicide resistance must be informed by an understanding of the ecological and evolutionary factors that drive selection in weed populations. Here, we argue for a greater focus on the ultimate causes of selection for resistance in herbicide resistance studies. The emerging fields of eco-evolutionary dynamics and applied evolutionary biology offer a means to achieve this goal and to consider herbicide resistance in a broader and sometimes novel context. Four relevant research questions are presented, which examine (i) the impact of herbicide dose on selection for resistance, (ii) plant fitness in herbicide resistance studies, (iii) the efficacy of herbicide rotations and mixtures and (iv) the impacts of gene flow on resistance evolution and spread. In all cases, fundamental ecology and evolution have the potential to offer new insights into herbicide resistance evolution and management.

Keywords: herbicide resistance; eco-evolutionary dynamics; evolutionary biology; selection; fitness; gene flow

1 INTRODUCTION

The study of contemporary evolution of resistance to herbicides in plant populations must be interdisciplinary, combining insights and approaches from plant molecular biology, physiology, genetics, ecology, evolutionary biology and agronomy. Over the last 30 years, a great deal of progress has been achieved in elucidating the physiological and molecular genetic mechanisms that result in the phenotypic expression of herbicide resistance.[1] Notwithstanding this, formidable challenges remain in this area, particularly with respect to complex mechanisms of non-target-site resistance. Advances in genomics and other 'omics' technologies offer exciting opportunities to enhance further current understanding of the complex array of defences that plants may evolve to withstand herbicides.[2] The adaptation of populations of weedy plants in the face of repeated exposure to herbicides is, however, fundamentally an eco-evolutionary phenomenon.[3–5]

In comparison with the wealth of studies that have focused on the physiological and molecular genetic basis of herbicide resistance, there have been relatively few explorations of the ecological and evolutionary processes that underpin evolution of resistance. There may be many reasons for this. In the 1960s and 1970s, weed biology and management were studied predominantly from a plant ecological perspective,[6–9] weeds being important model organisms in formulating the discipline of plant population biology.[10] However, the advent and unprecedented efficacy of chemical weed control transformed the study of weeds to one whose centre of gravity shifted towards plant physiology. As a consequence, perhaps the importance of ecological principles and their major role in understanding herbicide resistance have been underestimated.

Empirical studies of evolution in action are required to explore fully the eco-evolutionary dynamics of selection for resistance under different management regimes, and these studies are constrained by issues of temporal and spatial scale. Simulation modelling provides one means to overcome some of these limitations and challenges.[11] Our motivation in writing this article has been to consider the major economic problem of herbicide resistance in a broad eco-evolutionary context. Important research questions will be considered that it is believed can be illuminated by a greater focus on ecology and evolution. We hope to demonstrate that, far from being a problem particular to weed science, herbicide resistance is just one more example of rapid evolution in the face of human-influenced environmental change.[12] Recognition of this fact and collaboration with scientists working in applied evolutionary biology related to broader issues of climate change, conservation of biodiversity, biological invasions and healthcare can provide novel insight into herbicide resistance research.

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2 PROXIMATE AND ULTIMATE CAUSATION IN HERBICIDE RESISTANCE

In biology, distinctions are often made between the proximate and ultimate causes of biological function.[13,14] Proximate causation explains traits on the basis of the molecular and physiological mechanisms that result in their phenotypic expression. Ultimate explanations are more concerned with the ecological and evolutionary forces that act on those traits to increase the fitness of organisms expressing them. In the medical sciences, a greater focus on the distinction between proximate and ultimate causation in human health and disease has been an important consideration in the emerging discipline of evolutionary medicine.[15]

We believe these perspectives provide a powerful framework for reviewing approaches to the study of herbicide resistance. Understanding the molecular and physiological mechanisms of herbicide resistance is clearly important. Management strategies based on, for example, the rotation of herbicide modes of action are informed by knowledge of mechanisms of resistance. Knowledge of the molecular mechanisms of resistance can aid in the development of diagnostic tools,[16] and in future an intimate understanding of the diverse means by which plants can resist herbicides may aid in design of resistance-proof chemistry. Notwithstanding this, we would argue that currently there is too much focus on the *outcome* of selection for resistance in weedy plant populations (proximate causes) and a lack of emphasis on studies that seek to unravel the ecological and evolutionary *processes* that select for resistance (ultimate causes). Herbicide resistance research has enthusiastically adopted the *reductionist* paradigm and in doing so neglected more *holistic* approaches. Travisano and Shaw[17] have argued that the current emphasis on explaining the molecular detail of phenotypic traits is detracting from efforts to understand the ecological and evolutionary processes that result in phenotypic change. We believe this message has powerful resonance in relation to the study of herbicide resistance.

3 HERBICIDE RESISTANCE AS APPLIED EVOLUTIONARY BIOLOGY

There is an increasing recognition that ecological and evolutionary processes can occur on similar timescales,[12] such that rapid evolution has the potential to affect ecological processes and influence biological interactions over short timescales.[18] This phenomenon has been formalised in the framework of eco-evolutionary dynamics.[19] At the same time, it is now realised that much contemporary organismal evolution is being driven by human activity.[20,21] The novel discipline of applied evolutionary biology is emerging from these insights, and there has been a recent upsurge in publications and special journal issues that focus on human-directed evolution.[12]

The importance of the application of evolutionary principles to the management[22] of pressing economic, environmental and healthcare issues in medicine,[23] agriculture[24–26] and nature conservation[27] is becoming increasingly evident. In the realm of agriculture, it is clear that GM technology, pesticides, herbicides, biocontrol agents, invasive species, land use change and climate change are all causing evolution in the animal, plant and microbe communities associated with agroecosystems.[26]

Recently, the concept of evolutionary rescue has come to prominence in the wider literature[28] and in relation to the evolution of herbicide resistance.[5] Evolutionary rescue (ER) is based on the idea that, faced with novel and extreme environmental change, organisms may be able to evolve rapidly before populations decline to extinction. Essentially, weed populations have been rescuing themselves from the effects of herbicide toxicity for the last 40 years. In light of our contention that herbicide resistance research should adopt a more eco-evolutionary framework, these developments in the literature are exciting. They offer the potential to expand the context of our research and collaborate more widely with scientists from different disciplines who are grappling with problems that are driven by the same underlying processes.

4 STUDYING EVOLUTION IN ACTION

Although not all studies addressing the ecological and evolutionary context of herbicide resistance will require long-term selection experiments, these approaches will be important in order to study the *process* of selection for resistance. This is particularly true if a better understanding is to be gained, for example, of the impacts of relatively low and high herbicide use rates on evolution of resistance or the efficacy of herbicide rotations, sequences and mixtures to slow or even prevent evolution of resistance. The gold standard would be to perform replicated selection experiments on real weed populations at relevant population sizes and over long timescales. However, such experiments are beset with methodological and technical challenges: finding field sites with suitably herbicide-susceptible weed populations, securing funding for long-term and large-scale replicated field trials and preventing gene flow between populations in different treatment regimes and from neighbouring populations (to name a few). A number of field- and glasshouse-based selection experiments have attempted to study the evolutionary process of selection for resistance,[29–35] but few studies have been able to satisfy all of the requirements listed above.

Simulation modelling provides one means to overcome some of the challenges of conducting selection experiments, and the opportunities and constraints presented by this approach have been reviewed.[11] Studying the evolution of herbicide resistance in action in model plant species presents another opportunity. A few studies have utilised *Arabidopsis thaliana* as a model in selection experiments in herbicide resistance research, predominantly to explore the costs of resistance by tracking the frequency of known resistance alleles over time in the absence of selection.[36,37] Brotherton *et al.*[38] established differences in sensitivity to glyphosate between accessions of *A. thaliana* and tried (but failed) to select for decreased sensitivity. Others have used the single-celled chlorophyte *Chlamydomonas reinhardtii* as a model for exploring the evolutionary dynamics of selection for herbicide resistance.[39–41] The *Chlamydomonas* system has proved a useful tool for exploring the efficacy of resistance management prescriptions in delaying evolution of resistance, although questions remain about the translation of findings to higher plants.

5 THE DOSE RATE DEBATE

5.1 History and context

There has been a longstanding debate in the herbicide, pesticide and antimicrobial resistance literature about the propensity for reduced doses of xenobiotics to increase the rate at which resistance is selected.[42–46] In the context of the following discussion, a high rate is defined as being at or above the recommended use rate, and a low or reduced rate as somewhere below this rate.

In herbicide resistance, a full exploration of the rate debate requires recognition that, broadly, resistance can be selected either as a major gene or monogenic trait (for example, target-site resistance) or as a polygenic or quantitative trait (e.g. resistance based on enhanced herbicide metabolism). Gressel[42] has proposed the existence of a 'Catch-22' situation, whereby relatively high doses would select for major gene resistance and reduced application rates would select for quantitatively inherited resistance mechanisms. Gardner *et al.*[47] used a model to show that a revolving dose strategy could be an effective means to slow selection for both types of resistance. However, this strategy has never been tested empirically for herbicides.

There has been a similarly vociferous debate in the insecticide resistance literature.[44,48] Here, the weight of evidence suggests that quantitative resistance traits are rarely implicated in field-evolved insecticide resistance,[49] and therefore the potential for selection of polygenic resistance at low insecticide use rates is often discounted. However, notably, the high-dose refuge strategy has become a central pillar of resistance management in transgenic crops that produce *Bacillus thuringiensis* (*Bt*) toxins. This strategy is underpinned by the fact that high doses of the toxin will decrease the dominance of resistance traits and thus their heritability.[50] In a recent modelling study based on empirical data indicating a polygenic basis for resistance, Shi *et al.*[51] explored the impacts of dose consistency of fumigants in stored grain, concluding that survival of individuals at lower doses was a key driver of resistance evolution. In the field of fungicide resistance, most relevant studies have shown that selection for resistance is increased at higher doses.[46] Considering antibiotic resistance, Gullberg *et al.*[45] showed that antibiotic-resistant bacterial strains could be selected in natural (non-medical) environments following exposure of populations to doses of antibiotics up to several hundred-fold below the normal minimum inhibitory concentration.

Although remaining controversial, the dose rate debate clearly has huge practical significance, particularly in areas of the world such as Europe where legislation is moving to reduce pesticide application rates. Far from being an issue that is particular to the evolution of resistance to xenobiotics, the rate debate has parallels with fundamental questions in evolutionary biology and evolutionary ecology and can greatly benefit from insight from these areas of study.

5.2 Evolutionary genetics of the dose rate debate

Two fundamental and largely unanswered questions in evolutionary genetics are: Does most adaptation arise from novel mutations or from standing genetic variation within populations? Does adaptation proceed via fixation of single mutations of large effect or as a result of polygenic responses to selection?[52] These questions capture the essence of the dose rate debate, providing an excellent opportunity not only for evolutionary biology to illuminate a pressing practical issue in resistance management but also for research in herbicide resistance to inform basic evolutionary theory. Framed in the context of herbicide resistance, the questions become: Are responses to herbicide selection dominated by fixation of novel, major mutations (often single nucleotide polymorphisms in genes encoding herbicide targets) or does selection proceed more frequently by selection for, and recombination of, natural variation in plant defence pathways (non-target-site resistance)? Importantly, does the dose of herbicide applied influence which of these two modes of resistance is selected?

Although rarely demonstrated explicitly, it is reasonable to assume that weed populations with no prior exposure to herbicides will harbour additive genetic variation for herbicide sensitivity.[30,53] Anecdotal evidence to support this expectation is provided by herbicide dose–response assays on susceptible weed populations. Taking inspiration from McKenzie,[43] we propose a conceptual model where low herbicide doses will select for resistance if the dose applied acts within the range of standing phenotypic variation in sensitivity of the population (Fig. 1). In this situation, and where phenotypic variation is underpinned by additive genetic variation, recombination of this variation in an outcrossing species may lead to rapid increases in the resistance phenotype. In contrast, where insufficient additive genetic variation is available for selection, or where the dose of herbicide applied is beyond the range of standing variation, evolution of resistance may only proceed via the selection of novel mutations at major resistance genes. An important alternative explanation for low-dose-mediated evolution of resistance based on an epigenetic inheritance mechanism[54] cannot be discounted and

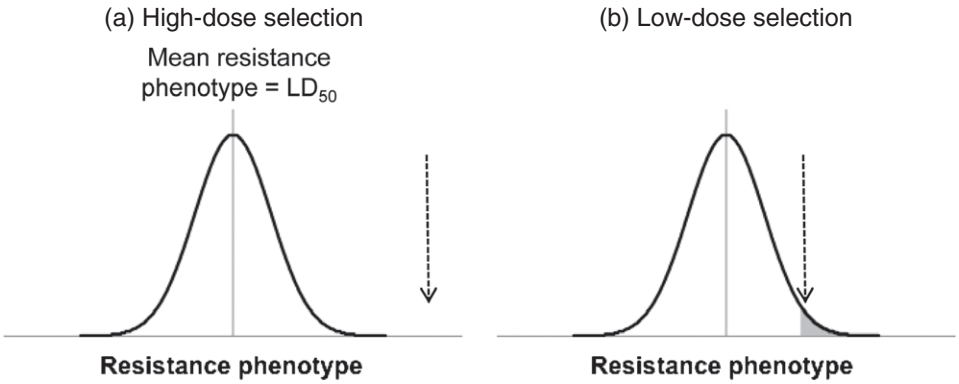

Figure 1. A conceptual model for (a) high-dose versus (b) low-dose herbicide resistance selection. The model assumes that unselected weed populations possess standing genetic variation for response to herbicides. This variation is represented by a normally distributed resistance phenotype (the resistance phenotype of an individual within a population being the minimum dose of herbicide that will cause mortality of that individual). The mean resistance phenotype is equivalent to the LD_{50} value for the population. Applied herbicide doses are shown with broken arrows. Where a high herbicide dose is applied (a), selection acts beyond the range of standing genetic variation and resistance can only evolve via selection of major resistance mutations that result in an extreme resistance phenotype. Where a low dose is applied (b), selection occurs within the range of standing variation. In outcrossing species, surviving individuals (grey shading) cross with one another, resulting in the selection and recombination of standing variation at minor resistance alleles.

is worthy of further research. There is mounting evidence that environmental stresses can elicit changes in DNA methylation patterns across the genome, resulting in changes in gene expression that could confer resistance to herbicides. The transgenerational inheritance of resistance-endowing epialleles[55] offers another intriguing explanation that may accelerate evolution of herbicide resistance,[1] particularly at low (sublethal) herbicide rates.

5.3 Evidence for low-dose herbicide selection

Neve and Powles[30] sought to test empirically the assumptions of the conceptual model described above. Using a well-characterised, susceptible population of *Lolium rigidum* (VLR1), a standard dose–response curve was derived for the population for the ACCase-inhibiting herbicide diclofop-methyl up to the field recommended dose in Australia (375 g AI ha^{-1}). The LD_{99} of the population was 300 g AI ha^{-1}. Twenty-eight individuals (36% of those treated) survived a dose rate of 37.5 g AI ha^{-1}. These individuals were grown to maturity and allowed to cross-pollinate (*L. rigidum* is an obligate outcrossing species). Seed was collected, and two further rounds of recurrent selection were performed. Dose–response assays were conducted on the third generation of low-dose selected VLR1, and large, significant increases in the resistance status of the population were observed; the LD_{50} of the 3 times selected seed population was 2.46 kg AI ha^{-1}, over 40 times the LD_{50} value estimated for the original susceptible population. Thus, a 40-fold increase in the resistance status of the population was achieved, based on selection of 28 individuals in the original population. Subsequent studies have confirmed that the mechanism of resistance selected was enhanced metabolism.[56]

Neve and Powles[30] clearly demonstrated proof of concept for low-dose herbicide selection. However, while the practice of rate cutting by farmers is widespread, selection for resistance in the field will rarely occur at 10% of the recommended rate. In a subsequent study, 31 populations of *L. rigidum* were collected from across sites in Australia where no herbicides had been previously applied.[57] The recommended rate of diclofop-methyl was applied to approximately 1000 individuals from each population. Survival ranged from 0 to 2.6%. For five populations, survivors were cross-pollinated to produce five discrete, once-selected seed populations. For all populations, dose–response assays revealed that the resistance present at high frequency was heritable. The mechanism of resistance was not based on target-site modification. Notably, one round of selection with diclofop-methyl had selected for diverse patterns of cross-resistance to a range of ACCase- and ALS-inhibiting herbicides. This study demonstrated that heritable additive genetic variation for resistance to diclofop-methyl is widespread in unselected *L. rigidum* populations, and that the genetic basis for this resistance is complex, as revealed by diverse patterns of cross-resistance. Clearly, the recommended use rate for diclofop-methyl in Australia selects within the range of standing genetic variation in *L. rigidum* populations.

These initial findings have motivated a range of other studies to explore low-dose selection. The potential for low-dose selection of resistance to diclofop-methyl in *L. rigidum* has now been demonstrated in the field.[34] Selection of the VLR1 population with reduced doses of glyphosate has been shown to result in an approximately twofold reduction in sensitivity,[31] and a multiply resistant population of *L. rigidum* exposed to below-recommended rates of pyroxasulfone evolved eightfold resistance to this newly commercialised herbicide.[35] Manalil *et al.*[53] adopted a novel approach to selecting for increased sensitivity to diclofop-methyl, and their observations provide further evidence for heritable variation in herbicide sensitivity in unselected weed populations. The potential for low herbicide doses to rapidly select for quantitative resistance has also been demonstrated using simulation models.[47,58]

Notwithstanding the mounting evidence that low herbicide doses can rapidly select for resistance, and recent demonstration that heritable variation in response to herbicides exists at high frequencies in some weed populations, it is not possible to generalise for all herbicides in all situations. A study by Beckie and Kirkland[32] showed that, in comparison with high application rates of ACCase-inhibiting herbicides in the field, low herbicide rates slowed increases in the frequency of *Avena fatua* individuals with known, major gene resistance. Similar dynamics have been demonstrated with simulation approaches[58,59] when resistance is endowed by a single, major gene. Although the genetics of low-dose selection are yet to be fully resolved (recombination of additive genetic variation or an epigenetic inheritance mechanism), a working hypothesis is that many alleles of minor effect are being recombined in outcrossing species to result in highly resistant phenotypes. A valid criticism of the emerging body of studies that explore evolution of herbicide resistance at low doses is that they have not directly compared low- and high-dose selection in the field. Until these studies are conducted, there is only compelling evidence that low-dose selection *can* occur, supported by modelling studies that suggest that evolution of quantitative resistance may occur more rapidly under low doses than under high doses even when major resistance genes are present in the population.[58]

5.4 Final thoughts on low-dose selection

There are at least two reasons why it might be expected that low-dose selection of quantitative resistance may be more of an issue in herbicide resistance than evidence suggests it to be in insecticide and fungicide resistance. Plants are sessile (aside from mobile seed and pollen phases), and this means that they have had to evolve a vast array of defences against abiotic and biotic stresses[60,61] as they are unable to escape unfavourable conditions. It is well known that there is a significant degree of crosstalk between plant defence pathways that mediate responses to these stresses.[62,63] In light of this, it may be speculated that weedy plants possess a degree of pre-adaptation in defence responses that can be selected upon exposure to herbicides. Secondly, in many cases, herbicide selectivity is based on the differential ability of crop and weed species to metabolise herbicides.[64,65] Hence, many weed species may be armed with low-level defences against herbicides prior to the imposition of any selection. Evolution of resistance simply requires them to evolve enhancements of physiological capacities that they already possess. Low doses make this easier to achieve, as they enable the survival of plants with an initially low level of resistance and the subsequent enhancement of the resistance phenotype through the recombination of variation at additive genetic loci. Finally, we offer one other perspective on the potential importance of unravelling responses to low herbicide doses. It seems clear, for some species at least, that high levels of (probably) metabolism-based resistance pre-exist in weed populations, sometimes at levels that allow individuals in previously unselected populations to survive. It is possible that during the early stages of selection for resistance it is this variation that is selected. Subsequently, as population sizes grow owing to the erosion of herbicide efficacy, the fixation of rare, major gene resistance may become more likely. Certainly, some emerging evidence in a large number of populations of *Alopecurus*

myosuroides is showing that target-site resistance to ACCase- and ALS-inhibiting herbicides is rarely present in a population without a background of enhanced metabolism (Knight C, unpublished data). These insights, if true, may provide solutions for herbicide resistance management.

6 PLANT FITNESS IN HERBICIDE RESISTANCE RESEARCH

6.1 Plant fitness and resistance evolution

The concept of fitness is fundamental to evolutionary biology.[66] Here, fitness is considered as the product of the probability of survival of individuals (or genotypes) comprising a population in a given environment and their fecundity. Fitness trade-offs (costs of resistance), driven by antagonistic pleiotropy, constrain evolutionary outcomes in variable environments and may often be expressed in herbicide-free environments. These resistance costs will moderate the rate of resistance evolution in heterogeneous environments (for example, with herbicide rotation) or may lead to a reduction in the frequency of resistance when selection is relaxed (cessation of herbicide treatment). In an agricultural environment, where herbicides may be rotated and/or used infrequently, resistance will evolve where the fitness advantage in the presence of herbicides (resistance benefit, RB) is greater than the resistance cost (RC).[67,68] The rate of resistance evolution will depend on the magnitude of the difference between *RB* and *RC* over the selective period.

6.2 Fitness in the presence of herbicides (RB)

The degree of resistance endowed by resistance mutations depends on their effectiveness in preventing the herbicide from reaching, binding to and inhibiting the target protein. However, the ultimate measure of resistance must consider the degree to which plants that survive herbicide application are able to maintain their reproductive fitness. A great deal of research effort has been invested in attempts to describe the level of resistance endowed by known resistance mechanisms, genes and alleles. Typically, these efforts focus on whole-plant dose response or enzyme inhibition assays to establish the impacts of a range of herbicide doses on plant mortality, plant growth during a short period following treatment or *in vitro* enzyme inhibition. Data produced from these assays can be analysed by non-linear regression to establish the herbicide dose required to achieve a 50% reduction in plant survival (LD_{50}), growth (GR_{50}) or enzyme inhibition (I_{50}). These studies provide a physiological measure of resistance (often called the resistance index) and provide important insights into the immediate effect of the resistance mechanism or allele on plant mortality under herbicide treatment. It is important to acknowledge, however, that, almost without fail, the fecundity of individuals following exposure to herbicides is not estimated, and therefore a true ecological measure of resistance[69] is not achieved.

In the field, the response to selection will depend on the relative number of resistant (R) and susceptible (S) survivors (determined by survival of the R genotype and escape from exposure of the S genotype), the dominance of the resistance trait, the dominance of the fitness cost and the relative fecundity of surviving R and S plants. Selection intensity has been identified as one of the most important parameters defining herbicide resistance evolution.[3,70,71] However, it is clear that inaccurate fitness estimations resulting from the lack of assessments of reproductive traits in R and S genotypes under herbicide selection will also lead to inaccurate estimations of selection intensity and thus rates of herbicide resistance evolution.

6.3 Fitness in the absence of herbicides (RC)

Theory predicts that there will often be a cost to adaptation.[72] However, resistance costs are not universal, and their expression has been shown to depend on the particular resistance mechanism,[73] the specific resistance allele,[74] the dominance of the resistance cost,[75] pleiotropic effects on the kinetics of herbicide target proteins,[76–78] genetic background[79] and environment.[67,68,80–83] The importance of fitness costs associated with herbicide resistance has been widely recognised and is evident in the wealth of studies that have endeavoured to establish costs of resistance. Estimating costs of resistance is challenging, and unfortunately the majority of studies in the literature have failed to employ suitable methodologies to establish unequivocally the presence and magnitude of costs of herbicide resistance.[67,68] The major limitations of these studies have been the failure to control for genetic background in which resistance traits are expressed and an absence of comprehensive measurements of fitness traits through the complete life cycle of the species. A number of methodological solutions have been proposed to address these shortcomings.[36,37,67,68,84]

6.4 Fitness and fitness costs in broad context

The widespread and repeated evolution of herbicide resistance in weeds provides ample evidence that resistance mutations provide large fitness benefits, and a mounting body of evidence suggests that resistance costs may range from moderate to relatively small.[68] Given this, is it important to conduct lengthy and methodologically challenging studies to estimate accurately the fitness benefits and costs of resistance mutations? We would argue that it is. These studies provide fundamental insight into the nature of adaptation to novel stresses. From a management perspective, understanding the life history trade-offs associated with resistance may open avenues for novel management. Accurately quantifying the fitness benefits of resistance mutations is important for modelling the evolution of resistance. In the future, rather than establishing plant responses to doses far in excess of those they will experience in the field, perhaps dose–response studies should determine more clearly the fitness of plants at field application rates. The concept of plant fitness has not been fully incorporated into herbicide resistance studies, and the challenge in doing so represents another area where basic evolutionary ecology has much to offer to the study of herbicide resistance.

7 ROTATIONS, SEQUENCES AND MIXTURES

Recommendations to rotate herbicide modes of action or to apply mixtures of different modes of action are a central pillar of resistance management.[5,85] The theoretical bases for these recommendations assume that resistance to different modes of action evolves independently. In the case of target-site resistance, where resistance is endowed by specific mutations at loci for herbicide target enzymes, these assumptions are well founded. Under this scenario, when herbicides A and B are rotated, there will be selection against resistance to herbicide B during exposure to herbicide A. Where there is a fitness cost associated with resistance to B, selection against this resistance during exposure to herbicide A will be more intense. Resistance to mixtures of modes of action may only evolve when resistance alleles to both mixture

components arise spontaneously in an individual, a phenomenon that occurs at very low frequency.

The concepts of specialism and generalism are central to ecological theory,[86] and the environmental and biotic determinants for the evolution and maintenance of specialism and generalism have been widely investigated.[87,88] Broadly, generalism is favoured by environmental heterogeneity. Target-site resistance represents a type of specialist resistance mechanism. Non-target-site resistance mechanisms often confer a more generalist resistance phenotype, such that selection of resistance to one herbicide mode of action confers broad-spectrum resistance to diverse modes of action.[1] Considering the two major modes of resistance evolution in these terms raises some interesting questions about potential unwanted side effects of herbicide rotations and mixtures. Rotation of herbicide modes of action is, after all, a form of environmental heterogeneity being experienced by weed populations. Could these strategies favour the evolution of generalist resistance mechanisms?

The potential benefits of herbicide rotations and mixtures have been demonstrated by a number of empirical[33,40,41] and theoretical[70,89,90] studies. However, most of these studies have considered the evolution of specialist resistance phenotypes, where selection for discrete resistance traits is independent. Using experimental evolutionary approaches in the unicellular chlorophyte *Chlamydomonas reinhardtii*, Lagator *et al.*[40] have shown that, for some herbicide combinations, rotation could accelerate evolution of resistance and result in the evolution of generalist phenotypes, resistant to herbicide modes of action that the populations had not previously been exposed to. Similarly, Lagator *et al.*[41] showed that mixtures of herbicides applied at low rates could select for generalist resistance. We are not foolish enough to suggest that herbicide rotations and mixtures do not have an important place in resistance management. However, the possibility for these much-promoted strategies to select preferentially for generalist resistance mechanisms should not be excluded. Here, once again, established theory from the field of evolutionary ecology that considers the impacts of environmental heterogeneity on the selection of specialist versus generalist resistance phenotypes has an important role to play in illuminating the debate about herbicide resistance management.

8 MIGRATION AND DISPERSAL

An evolutionary ecology framework has been employed to consider the impacts of genetic variation, selection, fitness and environmental heterogeneity on the evolution of herbicide resistance. Within this framework, the role of migration and dispersal of resistance alleles and phenotypes, via pollen and seed, in the evolution of herbicide resistance across agricultural landscapes must also be considered. In a broad sense, the key management question is whether resistance alleles migrate into susceptible weed populations at a rate greater than they arise via *de novo* mutation. The answer to this question has important implications in herbicide resistance management.[91] If the frequency of *in situ* mutation in a population (agricultural field) exceeds the rate at which resistance alleles migrate into that population, then the dynamics of resistance evolution will be primarily dictated according to management at the field level. If, on the other hand, migration from neighbouring resistant populations exceeds mutation rates, then evolution of resistance will be dictated by regional management practices.

A large number of studies have been conducted to quantify the extent of gene flow of herbicide resistance alleles between herbicide-resistant and non-resistant crop varieties and between herbicide-resistant and herbicide-susceptible weed populations. Rieger *et al.*[92] reported gene flow of ALS-resistance-conferring alleles between *Brassica napus* crops up to a distance of 3 km, although the frequency of gene flow between adjacent fields was much lower than 1%. Watrud *et al.*[93] found that the majority of gene flow from transgenic herbicide-resistant *Agrostis stolonifera* occurred within 2 km, although gene flow up to 21 km was observed. Pollen-mediated gene flow between herbicide-resistant and herbicide-susceptible populations of *L. rigidum* has been shown to occur to at least 3 km.[94] In another study, Busi *et al.*[95] found significant gene flow between conventional fields with herbicide-resistant populations of *L. rigidum* and neighbouring fields on organic farms with no history of herbicide exposure. Studying populations of highly selfing glyphosate-resistant *Conyza canadensis* in California, Okada *et al.*[96] found evidence of multiple independent origins of resistance. The weight of evidence suggests that considerable gene flow of resistance alleles can occur, probably at a frequency higher than background mutation rates. However, the actual contribution of *in situ* evolution versus migration-mediated evolution of resistance will probably depend on the interplay between demographic factors, source-sink dynamics and the local and regional scale of landscape and management heterogeneity.

In a study in France, Délye *et al.*[97] found evidence for very high frequencies of movement of herbicide resistance alleles between *Alopecurus myosuroides* populations in herbicide-treated fields and neighbouring organic fields. Délye *et al.*[97] inferred that small, well-controlled populations in organic fields were acting as sink populations for herbicide resistance alleles from adjacent dense, herbicide-resistant populations. This source-sink dynamic may have interesting parallels with the dynamics of biological invasion.[98] Source-sink dynamics is also thought to play a role in constraining adaptation in species range expansions.[99,100] As in previous case studies, evolutionary ecology may have something to say about the role of dispersal of herbicide resistance alleles on the evolution of herbicide resistance.

9 THE CASE FOR EVOLUTIONARY ECOLOGY

With this contribution, we have sought to justify their belief that herbicide resistance studies can benefit from a greater integration of ideas from fundamental ecology and evolution. In doing so, they do not underestimate the central importance of establishing the molecular and physiological basis of herbicide resistance. On the contrary, this understanding will underpin future attempts to conduct hypothesis-driven research that employs evolutionary thinking to improve herbicide resistance management. However, understanding the proximate causes of herbicide resistance should not preclude more holistic approaches in herbicide resistance research. The failure fully to integrate evolutionary biology into studies considering the evolution of resistance to antimicrobials has been noted by others.[101,102] Similar observations apply in herbicide resistance research,[103] and there is a great deal to be gained from closer collaboration with evolutionary biologists. The emerging discipline of applied evolutionary biology, which recognises the importance of rapid contemporary evolution in response to human activity, provides a framework in which to conduct these studies.[12] Indeed, agricultural weeds represent an excellent, economically important model species in which to study

rapid evolutionary responses to intense and sustained selection in a human-dominated ecosystem.[104]

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