Ecological and population genetics of herbicide resistance: where to now?

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Summary This paper reviews the current state of knowledge of the ecological and population genetics of herbicide resistance. We argue that increases in the understanding and ultimately management of resistance can be achieved by studying the processes which result in resistance, prior to, and during the early stages of evolution. In particular, we consider the level and nature of genetic variability for herbicide susceptibility in unselected weed populations, the dynamics of selection for resistance and the implications of pleiotropic fitness costs associated with resistant alleles. We conclude that many of the questions raised can be answered by research which is more focused on the population genetics which underpins resistance evolution. In particular, more attention should be given to related disciplines and more use made of emerging molecular tools.

Keywords Population genetics, herbicide resistance, genetic variability, fitness.

INTRODUCTION

The development of resistance to herbicides is an evolutionary phenomenon and, as such, it is underpinned by population genetic theory. The driving forces behind the dynamics of selection for herbicide resistance are well established, nevertheless, many of these remain poorly quantified. Understanding these processes and their inter-relationships has considerable merit on two levels. From a fundamental biological perspective, herbicide resistance represents a unique opportunity for studying microevolutionary processes in plants. From a more practical perspective, management of resistance cannot be uncoupled from underlying biological principles.

In most studies, the evolution of herbicide resistance has been studied retrospectively. We argue in this paper that significant advances in understanding and managing resistance can be achieved through research which is more focused on the processes and dynamics which occur in weed populations prior to, and during herbicide resistance evolution. This requires a greater comprehension of the population, ecological and genetic factors which underpin resistance evolution. In particular, lessons can be learnt, and principles applied, from closely related disciplines and research areas. These include insecticide and antibiotic resistance, evolution of tolerance to heavy metals in plants, the evolution of plant defence systems and general adaptations to stress. In addition, molecular tools and techniques are constantly being refined which have considerable utility in population genetics studies.

GENETIC VARIABILITY FOR HERBICIDE RESPONSE

Genetic variation between individuals of a species, and at a more localised level, between individuals in a population, is the raw material upon which natural selection acts to mould the evolution of newly adapted types. Within a population, microevolutionary forces select combinations of genes (genotypes) which produce highly adapted phenotypes. Environments, however, are not constant in either time or space and for this and other reasons (reviewed in Maynard-Smith, 1989), genetic variation is maintained within populations to enable them to respond to this environmental variability and to future challenges.

Clearly, the application of herbicides to weed populations constitutes a severe environmental perturbation. The ability of these populations to respond by producing herbicide resistant phenotypes relies on the existence of pre-existing genetic variability or the de novo production of variability.

Frequencies of resistance phenotypes in unselected weed populations Few contend that levels of variability for herbicide response in unselected (those which have never been exposed to the herbicide) weed populations are a major determinant of rates of, and propensities for herbicide resistance evolution. Often, this variability has been discussed, presented and modelled in terms of the initial gene frequency for resistance. This can be misleading as it assumes that there is a single mechanism, endowed by a single gene which confers resistance within the population, precluding the possibility of mechanisms which are controlled by genes at more than one loci or the existence of more than one mechanism. Unless, it is known that resistance in these unselected populations is due to a single mechanism controlled by a single gene, it is more accurate to talk in terms of the initial frequency of resistant phenotypes. As we discuss further the

dynamics of resistance evolution the importance of this consideration will become clearer.

The frequency of pre-existing resistant phenotypes in unselected weed populations is the product of many factors; mutation rates at loci which confer resistance, the number of loci capable of mutating to resistant alleles, the fitness of mutant alleles and phenotypes in the absence of herbicide selection, and gene flow between populations. Spontaneous mutation rates vary at different loci throughout the genome and may vary between populations of the same species. Typical rates have been reported as between 10^{-6} and 10^{-7} gametes per locus per generation (Maynard-Smith 1989). Few empirical studies have quantified the actual mutation rate to herbicide resistance in plant species. In *Arabidopsis thaliana*, mutations conferring target-site resistance to the acetolactate synthase inhibiting herbicides occurred at a frequency of approximately 1×10^{-9} (Haughn and Somerville 1987). In cell cultures of *Nicotiana tabacum*, mutation rates to primsulfuron resistance were estimated as 2.7×10^{-8} (Harms and DiMaio 1991).

In the absence of empirical data, most herbicide resistance models assume the initial frequency of resistance alleles to be 1×10^{-6}. There is, however, a mounting volume of data to suggest that in some species, initial frequencies of phenotypic resistance to some herbicides are far greater. Matthews (1996) found mean levels of resistance to diclofop-methyl applied at commercial rates (360 g a.i. ha^{-1}) of 0.5% in 27 unselected *Lolium rigidum* (annual ryegrass) populations from South Australia and Victoria. A similar study in Western Australia (Neve *et al.* unpublished data) demonstrated means levels of survival of 1.2%, 0.10%, 0.13% and 0.01% for diclofop-methyl, fluazifop-butyl, chlosulfuron and sulfometuron (at commercial rates), respectively in 15 unselected *L. rigidum* populations. Clearly, these results have major implications for rates of resistance evolution in Australian populations of annual ryegrass. The work reported has not, to date, attempted to establish the mechanisms and genetic control of resistance in these populations. This work is crucial if we are to understand the nature and origin of this variability, the evolutionary forces which maintain it in unselected populations, the relative fitness of these mutations and the dynamics of resistance evolution when herbicide selection is imposed. Such work will benefit greatly from biochemical and molecular diagnostic tools which will identify mechanisms and mutations and enable these to be tracked through populations once selection commences.

Response to sub-lethal rates Most studies have reported initial frequencies of resistant phenotypes when populations are screened with the recommended commercial rate of a herbicide. This is a rational approach as it closely mimics the selection regime which populations will face under field conditions. There is no reason to suspect, however, that these initial frequencies will not be dose specific. Matthews' (1996) 27 unselected ryegrass populations were also screened at half the recommended field rate of diclofop-methyl (180 g a.i. ha^{-1}) and mean survival was 2.8%, approximately five times greater than at the field rate.

These observations suggest quantitative (polygenic) variation in susceptibility to herbicides. Substantial quantitative variation for herbicide response has been reported in a number of other weed populations (see Jasieniuk *et al.* 1996). However, in most cases where the genetic control of field evolved resistance has been established this has been due to single genes (reviewed by Darmency 1994).

Notwithstanding this, Gressel has repeatedly argued that low rates of herbicides have the potential to select for polygenic or 'multifactorial' resistance in weed populations in the field (see Gressel 2002). Whilst evidence for this phenomenon remains scanty, there are a number of reasons why it deserves further attention. Herbicide resistance is usually studied 'after the fact' and researchers have, understandably, concentrated on the most resistant biotypes. There is no reason to suggest that the mechanisms of resistance we observe in highly evolved biotypes are the same as those that were selected in the early stages of evolution (Uyenoyama 1986). Indeed, Taylor and Feyereisen (1996) discuss the potential for genetic succession and the existence of pioneer and settler mutations in pesticide resistant insects. Additionally, work establishing the basis of inheritance of herbicide resistance should be interpreted with a degree of caution, as traits controlled by two, three or numerous loci may segregate in the same way as single gene traits in crossing experiments (Hoffman and Parsons 1991).

SOMATIC MUTATIONS, ADAPTIVE EVOLUTION AND HERBICIDE RESISTANCE

It is widely assumed that genetic variability for herbicide response must pre-exist in weed populations for resistance to evolve. Intuitively, this seems to be a simple and incontrovertible statement of Darwinian principles. However, it need not be true. In plants, unlike in animals, there is no real distinction between germline and somatic cells. Mutations which arise during DNA replication and cell division may proliferate during vegetative growth. Ultimately, cell lineages in which mutations have occurred may form gametes and

in this way somatic mutations can be passed from generation to generation. The role of somatic mutations in generating genetic variation in plants species has not been studied in great detail and not at all in relation to herbicide resistance.

What if herbicides actually increased rates of somatic mutations in plant cells ? Often such questions have been dismissed as advocating Lamarckian evolution under the misguided impression that they assume that herbicides cause mutations in the genes which code for their target sites – so-called directed mutation. There is a mounting body of evidence, particularly in bacteria (see Radman *et al.* 1999), that environmental stresses cause increases in rates of mutation and recombination. It is thought that this phenomenon is not directed (stresses do not initiate purposive mutations), but results in a general increase in mutation referred to as hyper- or adaptive mutation. Until recently, the genetic variation resulting from mutations, was thought to be an unavoidable stochastic error (Radman *et al.* 1999) brought about by the imperfection of DNA replication. More recently it has been recognised that in stressful environments, the ability to create de novo genetic variation may be adaptive and that mechanisms (alleles) which reduce the fidelity of DNA replication may be maintained at low frequencies in populations.

Similar phenomena have been observed in plants. Durrant (1962) reported the induction of heritable genetic change in flax under stressful conditions and McClintock (1984) showed how stressful conditions could increase the mobilisation of transposons and result in rapid genomic reorganisation in maize.

Darmency and Gasquez (1990) found evidence of individuals with herbicide resistance phenotypes intermediate between normal susceptible and resistant phenotypes (I types) in previously unselected populations of *Chenopodium album*. When triazine herbicides were applied at sub-lethal doses to these phenotypes they survived and produced progeny with dose responses characteristic of highly resistant individuals. These results hint at the existence of a subset of individuals within these populations that possess some sort of plastome mutator system which modulates mutation rates when individuals are environmentally challenged.

In higher animals, increased rates of mutation and recombination in immunoglobulin genes are targeted, well controlled and highly evolved (Weill and Reynauld 1996). These systems are responsible for the creation and maintenance of the somatic genetic variability which is essential to the immune system. Similar systems have been reported for other large gene families (see Caporale 2000). If we consider plants, cytochrome P450 monoxygeneases, glutathione-S-transferases and ABC transporters represent large gene families which perform roles broadly analogous to the immune system in animals. All have also been implicated to varying degrees in herbicide resistance. Could similar mutator systems have evolved in these gene families? If so, we may be able to explain the observation made for both insecticide (Gould 1995) and herbicide (Gressel 2002) resistance that the evolution of resistance to one chemical often predisposes individuals to more rapid evolution of resistance to further unrelated chemistries. Perhaps exposure to xenobiotics increases the frequency of these mutator systems in populations and thus increases the ability of insects and plants to adapt to novel challenges.

FITNESS COSTS, AMELIORATION AND FIELD REGRESSION

Studies attempting to identify and quantify fitness costs associated with herbicide resistance have often been hindered by methodological flaws and the inherent variability in weed populations which makes the detection of small differences in growth difficult.

Too often in the past, resistant and susceptible populations from different geographical locations have been compared. Where they are found, differences in growth and productivity cannot be attributed to the possession of resistance genes as growth traits are under complex genetic control which will have been subject to different selective forces in different environments. Measurement of fitness related traits in isogenic or near-isogenic lines has been advocated as a means of overcoming these difficulties. While such approaches have utility for identifying baseline physiological costs of resistance genes, they too are flawed at the practical level. Resistance genes in weed populations do not exist in a common genetic background and it is well known that the pleiotropic and epistatic effects of mutant alleles vary according to this genetic background. Over time, selection will favour combinations of genes which reduce these pleiotropic costs. In view of this, and where resistance is not fixed, the proper control for fitness studies may be susceptible individuals of the same population.

It is tempting in studies where apparent fitness costs are obscured by the inherent variability in growth traits to dismiss this 'noise' as nothing more than a nuisance. In fact, it is part of the result as it demonstrates that costs of resistance vary in different genetic backgrounds. As resistance becomes fixed in the population, selection will favour those genotypes in which pleiotropic costs are minimised. In effect, the pleiotropic costs can undergo evolution (Uyenoyama 1986). The potential for such resistance coevolution

in weeds has been given little attention, though it has been documented in antibiotic resistant bacteria living antibiotic-free environments (Lenski 1997).

Similar phenomena have also been reported in the insecticide resistance literature, where initial fitness costs are moderated by the evolution of modifier genes which offset these costs (McKenzie and Purvis 1984).

Finally, we have acknowledged previously that it is likely that a number of potential mechanisms for herbicide resistance exist in unselected populations. We should also be aware that the same mechanism may be due to different mutations of the same gene. These different mechanisms and mutations will (or will not) have their own fitness costs and the magnitude of these costs will determine which become fixed in populations. Molecular diagnostics has a key role to play in identifying these mutations and tracking their fate during resistance evolution.

A FINAL WORD

Herbicide resistance research and management can benefit from greater understanding and application of population genetics. In particular, research should focus more on the process and dynamics of resistance evolution and not solely on the results and consequences. To this end, much can be learnt from related disciplines. Molecular tools, capable of identifying particular mutations, will have great utility in studies which track the fate and frequency of mutations during resistance evolution.

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