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Fitness Costs Associated with Evolved Herbicide Resistance Alleles in Plants

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Summary

Predictions based on evolutionary theory suggest that the adaptive value of evolved herbicide resistance alleles may be compromised by the existence of fitness costs. There have been many studies to quantify fitness costs associated with novel herbicide resistance alleles, reflecting the importance of fitness costs for determining the evolutionary dynamics of resistance. However, many of these studies have incorrectly defined resistance or used inappropriate plant material and methods to measure fitness. This review has two major objectives. Firstly, to propose a methodological framework that establishes experimental criteria to unequivocally evaluate fitness costs. Our review then presents a comprehensive analysis of the literature on fitness costs associated with herbicide resistance alleles. This analysis reveals unquestionable evidence that some herbicide resistance alleles are associated with pleiotropic effects that result in plant fitness costs. Observed costs are evident from herbicide resistance endowing amino acid substitutions in proteins involved in amino acid, fatty acid, auxin and cellulose biosynthesis as well as enzymes involved in herbicide metabolism. However, these resistance fitness costs are not universal and their expression depends on particular plant alleles and mutations. The findings of this review are discussed within the context of the plant-defence trade off theory and herbicide resistance evolution.

I. Introduction

Weeds are a major constraint to crop production and herbicides are key elements of weed control in most global crop production systems. Herbicides applied to large populations of genetically diverse weedy plants exert a very strong selection pressure for evolution of resistance and evolved resistance in weed populations has been reported worldwide (Powles & Holtum, 1994; Powles & Shaner, 2001; Powles & Yu, 2010).

It is a basic tenet of evolutionary genetics that adaptation to a new environment will often have negative pleiotropic effects on fitness in the original environment, the so-called 'cost of adaptation' (Purrington, 2000; Strauss *et al.*, 2002). These fitness costs can prevent the fixation of novel adaptive alleles (Tian *et al.*, 2003) and contribute to the maintenance of genetic polymorphisms within populations (Antonovics & Thrall, 1994). However, as will be reviewed, it has been shown that some herbicide resistance alleles have no observable impact on plant fitness. An understanding of the fitness consequences of herbicide resistance alleles in the presence and absence of herbicide is important for predicting the evolutionary dynamics of herbicide resistance (Neve *et al.*, 2003) and, therefore, in conceiving strategies by which fitness costs can be manipulated to result in selection against resistance alleles (resistance management) (Jordan *et al.*, 1999; Friesen *et al.*, 2000; Beckie, 2006; Walsh & Powles, 2007).

Where a herbicide resistance allele confers a fitness cost there are at least three explanations for the origin of the fitness cost. Firstly, fitness costs may result when novel, resistance-conferring mutations in herbicide target enzymes (target-site resistance) also compromise or interfere at some level with normal plant function or metabolism (Cohan *et al.*, 1994; Groeters *et al.*, 1994; Chevillon *et al.*, 1995). For

example, a single amino acid substitution may cause a structural modification in the target enzyme that limits the herbicide binding but also compromises the efficiency of the enzyme function and kinetics (Tranel & Wright, 2002; Délye, 2005; Powles & Preston, 2006; Powles & Yu, 2010).

Secondly, resource-based allocation theory predicts a trade-off between plant reproduction, growth and defence functions (Coley *et al.*, 1985; Chapin III *et al.*, 1993; Herms & Mattson, 1994). Herbicide resistance is an evolved plant defence mechanism that could potentially divert resources away from growth and reproduction. For example, herbicide resistance endowed by enhanced metabolism may rely on the novel or increased production of cytochrome P450 enzymes (Werck-Reichhart *et al.*, 2000). According to the resource-based allocation model, where this novel enzyme production is constitutive, the additional energy and resource investments to synthesize these enzymes will divert resources away from growth and reproduction and may impose a resistance fitness cost in the absence of herbicide.

Thirdly, fitness costs may arise as a consequence of altered ecological interactions (Purrington, 2000; Strauss *et al.*, 2002). If a resistance allele has pleiotropic effects such that the resistant phenotype becomes, for instance, less attractive to pollinators or more susceptible to diseases, then fitness costs may occur independently or in addition to any energetic drain or alteration of normal metabolism (Salzmann *et al.*, 2008). It is emphasised that where there is a fitness cost associated with recently evolved herbicide resistance alleles co-adaptation and integration of the resistance allele into the genome can reduce the fitness cost over generations (Fisher, 1928).

Beyond their importance for informing herbicide resistance evolution and management, costs of evolved resistance to herbicides provide a good model for answering wider fundamental questions about the origin of the cost of plant adaptation. Additionally, understanding ecologically and evolutionarily based changes in plant resource acquisition and allocation patterns in response to environmental disturbance, stress and heterogeneity provides useful insights into the theory of weed community assembly (Weiher & Keddy, 1999), biodiversity and energy flow in agroecosystems (Swift & Anderson, 1994) as well as the potential of weed invasions into natural communities (Sax *et al.*, 2007).

There have been a wealth of studies unequivocally documenting a fitness cost associated with target-site resistance to triazine herbicides (Holt, 1990; Warwick, 1991; Gronwald, 1994; Holt & Thill, 1994; Bergelson & Purrington, 1996) and therefore, will not be further reviewed here. However, a few unique features of the effects of triazine resistance on fitness costs that have partly motivated the present review are worth mentioning. In the great majority of cases, evolved triazine resistance is endowed by a chloroplastic *psbA* gene mutation that encodes for a Ser-264-Gly amino acid substitution in the PSII D1 protein. This mutation endows resistance because it reduces the affinity of the binding site for triazine herbicides (Trebst, 1996) but also leads to a reduced photosynthetic capacity due to an inefficiency of electron transfer within the PSII complex (Jansen & Pfister, 1990). As a result, many triazine resistant weeds possessing the Ser-264-Gly mutation show significantly reduced photosynthetic potential, growth rates, resource competitive ability and sexual reproduction (reviewed by Holt & Thill, 1994). It is understandable that the light reactions of photosynthesis have been optimised over evolutionary time and that the triazine resistance endowing mutation reduces the

efficiency of the light reactions of photosynthesis. Additionally, an interesting aspect of the Ser-264-Gly mutation is that the expression and magnitude of its associated fitness cost has been shown to be modulated by abiotic and biotic factors. Contrasting combinations of light and temperature may amplify, neutralize or even reverse its negative effect on photosynthesis and plant growth (Ducruet & Ort, 1988; Hart & Stemler, 1990; Dekker & Sharkey, 1992; Plowman & Richards, 1997; Arntz *et al.*, 1998; Jordan *et al.*, 1999; Plowman *et al.*, 1999; Arntz *et al.*, 2000). Similarly, triazine resistant plants are more susceptible to fungal infections and insect herbivory, further contributing to the fitness cost of the resistance endowing Ser-264-Gly mutation (Gassmann, 2005; Gassmann & Futuyma, 2005; Salzmann *et al.*, 2008). The increased preference of herbivores for triazine resistant plants has been shown to correlate with higher concentrations of nitrogen in leaf tissues. It is speculated that the increase in leaf-level nitrogen concentration is a potentially photosynthetic compensatory trait which ultimately translates into an ecologically based cost of greater susceptibility to herbivores and consequently into a significant higher fitness cost associated with triazine resistance (Gassmann, 2005).

The magnitude of costs associated with triazine resistance has meant that these costs have been easily detected, despite the often flawed methodologies that have been employed to measure fitness (Bergelson & Purrington, 1996; Jasieniuk *et al.*, 1996). A major motivation for this review is to highlight that the triazine-resistance example of near universality of a single mutation with a strong fitness cost is the exception rather than the rule. As will be fully elucidated in the Sections that follow, there is mounting evidence for the existence of a plethora of herbicide resistance mechanisms and mutations, some of which may lead to pleiotropic effects with negative, positive or neutral consequences on plant fitness.

The purpose of this article is twofold; to consider the methodological and experimental requirements to unequivocally measure fitness and fitness costs in plant populations and secondly, to review evidence from published studies for costs of resistance associated with non triazine herbicides.

To achieve these objectives, this review critically examines molecular, physiological and ecological factors influencing the expression and magnitude of fitness costs associated with evolved resistance to non-triazine herbicides in weedy plant species. A total of 55 published articles have been identified in citation databases (Scopus® and Web of Science®) (Supplementary Material Appendix S1). We provide a critical analysis of the experimental methodologies that have been used to assess the expression and magnitude of fitness costs and their correlation with the physiological/molecular bases of herbicide resistance.

II. Methods for the detection and estimation of herbicide resistance fitness costs

We propose five experimental factors that should be considered in the design of studies to estimate and interpret fitness costs associated with resistance to herbicides. In this section, we will present the rationale for considering each of these factors before reviewing the extent to which each of them have been considered in published fitness studies.

1. Control of genetic background

In response to the varying abiotic and biotic selective forces, ecotypic differentiation is a common process occurring at different spatial scales among weed species (Snaydon, 1971; Ransom *et al.*, 1998; Keller & Kollmann, 1999). Thus herbicide resistant and susceptible individuals from different plant populations will likely

exhibit genetic variability at a number of fitness-related loci (Bergelson & Purrington, 1996; Jasieniuk *et al.*, 1996). Therefore, to unequivocally attribute costs to the herbicide resistance endowing allele, relative fitness should be measured in resistant and susceptible individuals that share a similar genotype except for the alleles endowing herbicide resistance.

More than a decade ago, it was concluded that numerous studies assessing herbicide resistance fitness costs (most of them on triazine resistance) were flawed as they did not minimise differences in the genetic background between resistant and susceptible populations or individuals (Bergelson & Purrington, 1996). Our literature review reveals that only 25% of studies assessing fitness costs explicitly met the criteria of control of genetic background. Among the studies that did control genetic background, the experimental protocols to create suitable herbicide resistant and susceptible lines for comparison included genetic transformation, production of segregating F2 lines and identification of co-segregating herbicide resistant and susceptible individuals from within single populations (Purrington & Bergelson, 1997; Roux *et al.*, 2004; Vila-Aiub *et al.*, 2005a; Menchari *et al.*, 2008).

An alternative approach may be to reduce the effect of differences in genetic background by comparing multiple resistant and susceptible populations (Cousens *et al.*, 1997; Strauss *et al.*, 2002). When multiple comparisons between resistant and susceptible genotypes are also considered, the proportion of the studies satisfying the control of genetic background criterion rises to about half of the published literature.

2. Knowledge of the biochemical basis of herbicide resistance

Where possible it is desirable to have characterized the biochemical and molecular basis of resistance before conducting a fitness study. This knowledge is essential to ascribe identified pleiotropic effects to particular gene and mutations and comprehend their biochemical and physiological origin and causes.

The importance of this proposed experimental requirement is evident as too often more than one resistance endowing mechanism may be present at the population and individual level and different resistance mutations may be involved. For example, it is now well established that either or both enhanced detoxification and an insensitive target enzyme may endow resistance to acetyl-CoA carboxylase (ACCase) or acetohydroxyacid synthase (AHAS) inhibiting herbicides in *Alopecurus myosuroides* and *Lolium rigidum* populations (Tardif & Powles, 1994; Preston, 2004; Powles & Yu, 2010). Moreover, for both target site ACCase and AHAS herbicide resistance there are several different mutations (Tranel & Wright, 2002; Délye, 2005; Yu *et al.*, 2007a), some associated with a fitness cost (Roux *et al.*, 2004; Menchari *et al.*, 2008) and others not (Vila-Aiub *et al.*, 2005a). Given this reality, the almost 50% of published studies that evaluated fitness costs without knowledge of the biochemical/molecular basis of resistance have evident limitations.

Although their limitations in understanding the biochemical origin of fitness cost, it should be also emphasized that proper fitness studies can be conducted in which the molecular and biochemical bases of resistance are yet to be identified. Results from these experiments may be useful for the design of weed management strategies to exploit those traits that result in reduced ecological performance. This is the case for triallate and difenzoquat herbicide resistant *Avena fatua* populations that exhibit significant higher germination rates when compared to susceptible genotypes

(O'Donovan *et al.*, 1999). Significant differences (or lack of them) (Murphy *et al.*, 1986) in life history weed traits should provide knowledge for the adoption of agronomic tools to manage herbicide resistant populations.

Herbicide resistance mechanisms can be broadly divided into target-site and non target-site mechanisms. The majority of fitness cost studies in the literature are on target-site herbicide resistance. However, while there are many cases of non-target site herbicide resistance (Preston, 2004; Preston & Wakelin, 2008) there are thus-far only few studies of fitness costs of non target site resistance mechanisms (see Sections VII and VIII).

In total, only 28% of fitness studies combine knowledge of the molecular basis of resistance with adequate control of genetic background (Supplementary Material Appendix S1). These 28% of studies are valuable in fully understanding herbicide resistance fitness costs in plants.

3. Life history traits

Plant fitness may be defined as the relative number of offspring contributed to future generations by one form compared to others (Harper, 1977; Primack & Hyesoon, 1989). This focus on plant reproduction as a measure of fitness has led to many fitness studies that compare seed production between herbicide resistant and susceptible individuals. However, the assumption that a genotype that produces many seeds is fitter than a genotype producing fewer seeds is only true if seed dispersal, germination and colonization rates, seed longevity, seedling vigour and resistance to pathogens, diseases or herbivores are identical (Primack & Hyesoon, 1989; Hanley, 1998). Also, calculating the number of seeds produced by an individual only estimates its female reproductive fitness, when in fact the number of

copies of a resistance allele that are transmitted to the next generation is the sum of male and female reproductive fitness. For example, in an outcrossing species, estimates of seed production of resistant plants may considerably overestimate fitness of the resistance allele if female gametes are preferentially pollinated by neighbouring susceptible plants. Most fitness studies which measure seed production do not reveal if they account for male reproductive fitness. A number of potential ways to account for male reproductive fitness are possible such as evaluation of the production, viability, growth and competition between resistant and susceptible pollen (Delph *et al.*, 1998; Song *et al.*, 2002), and pollen discounting in species with both selfing and outcrossing reproductive systems (Chang & Rausher, 1998). Similarly, ovule size and number may be also estimated to evaluate female reproductive fitness (Burd *et al.*, 2009). Some methodological protocols such as prevention of cross-pollination between resistant and susceptible plants (Vila-Aiub *et al.*, 2009) and genotypic determination of seeds produced in mixed resistant and susceptible populations (Roux *et al.*, 2005a) are designed to minimize the confounding potential differences in male and female reproductive fitness when assessing costs between herbicide resistant and susceptible genotypes.

The reality is that there are trade-offs between different life history stages and changes in one component of plant fitness may involve compromises in other traits (Harper, 1977). Seed production is a crucial determinant of fitness which will integrate and be influenced by other life history variation between resistant and susceptible plants. However, it remains important to understand how resistance impacts on other life history processes, as this knowledge may be applied to the design of appropriate weed management to maximise fitness costs. The majority of studies inferred costs associated with resistance alleles after exploring phenological,

morphological, physiological and growth plant traits at the vegetative and/or reproductive stages. Exploration of expression of fitness costs and life history variation associated with traits and processes that occur early in the plant life cycle are important as any fitness costs evident in young plants will have significant adverse impact in a competitive world. Of a total of 112 experiments (replicated experiments were counted as one) identified in the reviewed literature, only 7% and 10% evaluated the occurrence of fitness costs during seed germination and plant establishment, respectively.

We believe that the best indication of the fitness of individuals carrying a resistance allele is evident when the frequency of the given allele or genotype is followed over several generations (Roux *et al.*, 2005a; Roux *et al.*, 2006; Wang *et al.*, 2009). Observed variations in the resistance allele frequency are compared to an expected allele frequency given no fitness costs and no genetic drift. A similar methodological approach involves the assessment of the resistant phenotypic frequencies after a certain time of discontinued herbicide use (Bourdôt *et al.*, 1996; Andrews & Morrison, 1997). The “allele frequency approach” enables the evaluation of the evolutionary trajectories of resistance genes without identifying the particular plant traits involved, account for male and female reproductive fitness and, by default, integrates fitness effects across all life history stages. This experimental protocol has proved to be a robust tool to assess fitness costs associated with insecticide (Boivin *et al.*, 2003; Hardstone *et al.*, 2009) and antibiotic (Gustafsson *et al.*, 2003) resistance, and can be extended to the study of fitness costs associated with polygenic traits and traits of unknown biochemical resistance mechanism. In this regard, studies which are conducted in the field in

appropriate natural environments can best simulate the conditions in which fitness costs express in agroecosystems.

4. Resource competition

Ecological fitness costs are differentially expressed depending on interactions with other organisms (Strauss *et al.*, 2002). Ecological costs may become evident as a consequence of biotic interactions such as predation (Gassmann, 2005), disease (Brown, 2003) and/or competition (Van Dam & Baldwin, 2001). If a herbicide resistance allele results in impaired ability to capture resources or less efficient use of captured resources, then ecological fitness costs should be more evident under intense resource competitive conditions. For this reason, it is easier to measure herbicide resistance fitness costs under competitive conditions (Harper, 1977; Weiner, 1990; Reboud & Till-Bottraud, 1991; van Dam & Baldwin, 1998; Glawe *et al.*, 2003).

Around half of the 55 reviewed studies have measured fitness costs under competitive conditions. Unfortunately, the results of many of these studies are inconclusive and difficult to interpret as there was no control of genetic background. Interestingly, in studies whose experimental design involved both plant resource competitive conditions and control of the genetic background between compared resistant and susceptible ecotypes, expression of fitness costs was almost always reported (Roux *et al.*, 2004; Tardif *et al.*, 2006; Pedersen *et al.*, 2007; Menchari *et al.*, 2008; Vila-Aiub *et al.*, 2009).

5. Environmental gradient

The expression and magnitude of fitness costs associated with resistance alleles may be environment-specific (Heidel *et al.*, 2004; Martin & Lenormand, 2006; Jessup & Bohannan, 2008). There is a general premise that fitness costs may be more evident when plants are growing under more extreme, stressful environmental conditions (Coley *et al.*, 1985). Although this prediction is not always true (Bergelson & Purrington, 1996; Marak *et al.*, 2003), it may be more relevant when a mechanism endowing herbicide resistance depends on limited environmental resources to operate. For instance, if resistance depends on herbicide sequestration or detoxification, or cellular processes that require the synthesis of constitutively expressed N-rich proteins, then plant growth may be compromised in nitrogen poor environments (Lerdau & Gershenzon, 1997; Baldwin *et al.*, 1998). This knowledge may help managers to "design environments that potentially exploit functional weaknesses of herbicide resistant weeds" (Jordan *et al.*, 1999).

The effects of temperature on the expression of fitness costs have been studied more than any other environmental factor. However, potential temperature-regulated costs have largely been focused on seed germination responses. This is the case for AHAS resistance alleles which have shown a strong correlation with higher germination rates at cool environments (Dyer *et al.*, 1993; Thompson *et al.*, 1994; Park *et al.*, 2004) (see Section III). Overall, most fitness cost studies have not considered environmental variables as they have mostly been in controlled environment conditions, often optimal for growth. A few publications have aimed to assess herbicide resistance related fitness costs under contrasting light, nutrient and water conditions (Purrington & Bergelson, 1997; Ismail *et al.*, 2002; Vila-Aiub *et al.*, 2005b; Menalled & Smith, 2007; Menchari *et al.*, 2008). When control of

genetic background was carried out, these reports have recognised a significant impact of the environment on the expression of fitness costs (see sections III and IV).

In the following sections we have distilled the literature on the expression and magnitude of fitness costs associated with herbicide resistance alleles. We focus on studies that satisfy the requirements of control of genetic background and knowledge of the specific resistance mechanisms in the plants under study (other studies that corroborate findings in these studies are also discussed). When possible, if not reported, the magnitude of the fitness cost (%) was calculated as [1 – (resistant plant descriptor / susceptible plant descriptor) x 100].

III. Fitness costs associated with AHAS target site resistance

AHAS inhibiting herbicides inhibit acetohydroxyacid synthase (AHAS, also extensively referred to as ALS), a key plastidic enzyme responsible for the synthesis of isoleucine, valine and leucine amino acids. In response to widespread use of AHAS inhibiting herbicides, many weed species have evolved target-site based resistance, due to mutations of the AHAS gene (reviewed by Saari *et al.*, 1994; Tranel & Wright, 2002; Powles & Yu, 2010). Resistance-endowing mutations at seven key amino acid residues (Ala-122, Pro-197, Ala-205, Asp-376, Trp-574, Ser-653 and Gly-654) in the AHAS gene have been documented (Table 1A, B).

It is evident from studies with several resistant weed species that Pro-197 can be substituted by several amino acids to result in AHAS herbicide resistance (Yu *et al.*, 2007b; Tranel *et al.*, 2008; Yu *et al.*, 2008) (Table 1A, B). Fitness consequences have only been examined for the Pro-197-His substitution. A field study examining changes in the phenotypic frequency of field evolved resistant *Lactuca serriola*

individuals possessing the Pro-197-His allele (Guttieri *et al.*, 1992) showed a decline of between 25% and 86% over three years (Alcocer-Ruthling *et al.*, 1992a). This fitness cost was evident as a 15%[1] reduction in vegetative biomass of resistant compared to susceptible *L. serriola* individuals growing under competitive conditions (Alcocer-Ruthling *et al.*, 1992b).

Strong pleiotropic effects on plant morphology and anatomy leading to a fitness cost were described in field evolved AHAS resistant *A. powellii* with the Trp-574-Leu AHAS mutation (Tardif *et al.*, 2006). An examination of several resistant *A. powelli* populations revealed that this mutation was associated with thinner roots and stems and a severe leaf area reduction which led to a resistance cost of 67% (aboveground vegetative biomass) as well as a severe reduction in seed production (Tardif *et al.*, 2006). Further evidence for a cost associated with AHAS resistance comes from imidazolinone resistant rice crops with the Gly-654-Glu AHAS mutation which show 5-11% lower grain yield when compared to conventional rice cultivars (Sha *et al.*, 2007).

For target site resistance, an amino acid substitution in the herbicide target site enzyme prevents herbicide binding. The mutation may also impair enzyme activity and/or reduce substrate affinity (resulting in insufficient product biosynthesis). Indeed, impaired AHAS activity appears to be the case for the Pro-197-His substitution in *L. serriola* and *L. sativa*, and the Ala-205-Val substitution in *Solanum ptychanthum*. In both cases, the resistant AHAS shows no altered substrate binding but a near 50% reduction in activity compared to the wild type (Eberlein *et al.*, 1997; Eberlein *et al.*, 1999; Ashigh & Tardif, 2007). Similarly, in yeast Ala-122-

[1] Estimated as average over planting mixtures with equally proportion ratios (50:50, 100:100, 150:150)

Val and Ala-205-Val (Duggleby *et al.*, 2003) and tobacco Trp-574-Phe (Chong *et al.*, 1999) AHAS resistance substitutions result in a reduction in AHAS activity and substrate affinity. However, for other AHAS mutations there is no evidence of a reduction in AHAS activity with reports of no change in AHAS activity (Pro-197-Thr) (Preston *et al.*, 2006) or, in other cases, significantly higher AHAS activity reported (Pro-197-Ser, Trp-574-Leu) (Boutsalis *et al.*, 1999; Purrington & Bergelson, 1999; Yu *et al.*, 2003) (but see Mourad *et al.*, 1995). Obviously, the impact of each specific mutation/amino acid substitution needs to be evaluated on a case-by-case basis and generalisations should not be made.

AHAS activity is feedback regulated by accumulation of end-product branched-chain amino acids (Miflin & Cave, 1972). Whereas for the Pro-197-Ser substitution contradictory reports may be found (Tourneur *et al.*, 1993; Mourad *et al.*, 1995; Purrington & Bergelson, 1999), it is clear that the Pro-197-His (Eberlein *et al.*, 1997), Pro-197-Thr (Preston *et al.*, 2006) and Ala-205-Val (Ashigh & Tardif, 2007) AHAS mutations lead to a decreased sensitivity to feedback inhibition by valine, leucine and/or isoleucine.

A possible consequence of reduced feedback sensitivity to inhibition is the accumulation of branched-chain amino acids in plant tissues. An excess and/or imbalance of the amino acid pool can have toxic effects on cell metabolism and correlates well with diminished plant growth (Höfgen *et al.*, 1995). Higher concentrations of valine, leucine and isoleucine have been reported in leaves and seeds of plant species carrying AHAS mutations at the Pro-197 residue (Dyer *et al.*, 1993; Eberlein *et al.*, 1999; Purrington & Bergelson, 1999). Interestingly, this higher concentration of free amino acids is correlated with higher seed germination rates at relatively low temperatures (Dyer *et al.*, 1993). Rapid germination at cool

temperatures seems to be a characteristic trait associated with AHAS target site resistance (Pro-197-Ser/Arg/Thr), which, depending on the prevailing agroecological conditions, could turn into either a fitness advantage or disadvantage (Thompson *et al.*, 1994; Park *et al.*, 2004). Despite these reports, no published studies have examined and linked this particular germination response with seedling emergence and establishment processes.

Thus, it is clear that the His-197 mutation in *L. serriola* and the Leu-574 mutation in *A. powellii* are associated with a fitness cost (Alcocer-Ruthling *et al.*, 1992a; Alcocer-Ruthling *et al.*, 1992b; Tardif *et al.*, 2006). The origin of this cost may be due to either decreased AHAS activity or reduced feedback inhibition. These two explanations have compensating effects in terms of the quantity of branched-chain amino acid synthesis and further research is required to discern the main driving factor for these fitness costs. For the Leu-574 mutation, no AHAS data is available. For other resistance endowing AHAS mutations listed in Table 1B, their impact on plant fitness remains unknown and these studies are required. Additionally, it is also important to emphasise that, especially in *Lolium*, resistant plants frequently have a combination of two different resistance endowing AHAS alleles (Yu *et al.*, 2008) and fitness studies have not been conducted with such multiple field selected resistant plants.

IV. Fitness costs associated with ACCase target site resistance

In the majority of monocot (but not dicot) species, the ACCase herbicides are potent inhibitors of the key plastidic enzyme, acetyl-coenzyme A carboxylase (ACCase). Inhibition of ACCase results in a lethal disruption of fatty acid synthesis and many grass weed species have evolved ACCase herbicide resistance due to reduced

herbicide sensitivity of ACCase (Devine & Shimabukuro, 1994; Délye, 2005). To date, eight amino acid substitutions have been shown to confer ACCase resistance in field-evolved weeds (Table 2A, B) (reviewed by Délye, 2005; Liu *et al.*, 2007; Yu *et al.*, 2007a; Powles & Yu, 2010).

The resistance endowing Ile-1781-Leu substitution, has evolved in several weed species (reviewed by Délye, 2005; Zhang & Powles, 2006; Délye *et al.*, 2007; Yu *et al.*, 2007a; Powles & Yu, 2010). This amino acid substitution does not impair ACCase function (Shukla *et al.*, 1997; Délye *et al.*, 2002; Yu *et al.*, 2007a). Studies evaluating several fitness-related traits in *L. rigidum* and *A. myosuroides* with the Ile-1781-Leu mutation show no physiological or ecological resistance costs and no detectable reduction in plant productivity (Vila-Aiub *et al.*, 2005a; Vila-Aiub *et al.*, 2005b; Menchari *et al.*, 2008). Interestingly, when introgressed into a *S. italica* population the Ile-1781-Leu resistance gene has been shown to correlate with an increase in several fitness components, resulting in an increase in frequency of resistant genotypes over time (Wang & Darmency, 1997; Wang *et al.*, 2009).

In one *L. rigidum* population, the Ile-1781-Leu ACCase mutation has been shown to be associated with a strong environmentally-determined dormancy in seed, so that germination and emergence is promoted by light and fluctuating temperatures (Vila-Aiub *et al.*, 2005b). Although this modification of germination dynamics does not represent a fitness cost *per se*, it may be exploited by agronomic practices to reduce weed infestations in cropping systems. While this phenotypic response has proved to be a heritable trait, its occurrence across other genetic backgrounds needs to be assessed.

Menchari et al (2008) evaluated fitness in several *A. myosuroides* populations with Ile-2041-Asn and the Asp-2078-Gly ACCase resistance-endowing

mutations. In a two year field experiment where plants were grown in competition with wheat, there was clear evidence that the Ile-2041-Asn was not associated with any adverse pleiotropic effects on vegetative and reproductive plant traits. However, for individuals with the Asp-2078-Gly mutation, substantially impaired growth was observed when plants were grown with a limited water supply. Individuals possessing the Asp-2078-Gly mutation displayed a reduction in height (6%) and vegetative (42%) and reproductive biomass (36%) when compared to the wild type. However, this significant resistance cost was only observed in homozygote resistant plants (2078-Gly/2078-Gly) indicating that the fitness cost is recessive and environment dependent (Menchari *et al.*, 2008). Similarly, the 2078-Gly resistance mutation in the homozygous state has adverse pleiotropic effects on resistant *L. rigidum* growth (Vila-Aiub and Powles, unpublished data).

Moderate and strong reduction in ACCase-specific activity has been reported for the Ile-2041-Asn and Asp-2078-Gly mutations, respectively (Délye *et al.*, 2003; Délye *et al.*, 2005; Yu *et al.*, 2007a). The greatly reduced ACCase activity in plants with the Asp-2078-Gly mutations is likely to explain the fitness cost of this mutation. Recently, a new ACCase resistance mutation (Cys-2088-Arg) has been identified in *L. rigidum* (Yu *et al.*, 2007a). The ACCase activity of plants with this mutation is only half that of the wild type plants (Yu *et al.*, 2007a) and this impaired ACCase activity is likely responsible for the compromised growth observed at the whole plant level (Vila-Aiub and Powles, unpublished data).

The results reviewed above clearly show that the expression of fitness costs associated with ACCase resistance alleles is specific to the amino acid substitution conferring resistance, with different resistance substitutions in the same gene impacting very differently on enzyme functionality and associated pleiotropic

effects. For several of the resistance endowing ACCase resistance alleles, fitness studies have yet to be performed (Table 2B).

V. Fitness costs associated with resistance to phenoxy herbicides

Although the precise mode of action of these phenoxy type herbicides remains unknown, they clearly disrupt endogenous auxin metabolism (reviewed by Coupland, 1994) and for some field-selected resistant weed species there is a reduction in the binding affinity of these herbicides to auxin-binding proteins (Webb & Hall, 1995; Deshpande & Hall, 2000). However, despite decades of use, there have few cases of evolved resistance to phenoxy herbicides (reviewed by Coupland, 1994). As a result, there are few unequivocal fitness studies in plant species that have evolved resistance to the phenoxy herbicides. In a field study conducted in New Zealand, the frequency of MCPA resistant *Ranunculus acris* individuals declined by approximately 5% per year over five years (Bourdôt *et al.*, 1996). This study also reported a concurrent decline of about 50% in the LD_{50} (1.41 kg MCPA ha^{-1}) for a population removed from MCPA selection, in comparison with a *R. acris* population maintained under MCPA selection (LD_{50} = 2.13 kg MCPA ha^{-1}). The molecular basis of resistance in this field-selected population has not been reported.

Investigating a *Sinapsis arvensis* population with evolved resistance to various phenoxy herbicides (2,4-D, dicamba, MCPA and picloram), Hall and Romano (1995) report numerous pleiotropic effects on plant morphology and physiology. The resistant genotype showed a significant reduction in resource acquisition leading to short and small plants with reduced leaf area and a less developed root system (Hall & Romano, 1995). Furthermore, higher chlorophyll and cytokinin levels were associated with resistance to auxinic herbicides in this species.

The resistance gene/s endowing auxinic herbicide resistance in *S. arvensis* have not been elucidated and potential confounding effects derived from using plant material with different genetic background are possible. However, the reported pleiotropic effects are similar to those observed in *A. thaliana* carrying the AXR1 resistance gene (see next section).

VI. *Arabidopsis thaliana* as a model plant to understand pleiotropic effects of herbicide target-site based resistance alleles

The model plant, *Arabidopsis thaliana*, presents a number of intriguing opportunities to study the costs associated with herbicide resistance alleles. Known resistance alleles may be transgenically inserted into a standard genetic background or EMS-mutagenesis may be used to create herbicide resistant mutant lines. Fitness can be measured by assessing seed production of transgenic, mutant and wild type lines and high throughput genotyping can be used to confirm the genotype of individual plants. Results from these studies, together with a consideration of the advantages and limitations of these approaches are reviewed here.

There have been attempts to determine the fitness costs associated with the Pro-197-Ser AHAS mutation in transgenic and EMS mutagenised *A. thaliana* plants. Transgenic plants carrying this mutant allele display a reduction (26-34%) in the number of fruits per plant but not in total plant biomass or seed germinability (Bergelson *et al.*, 1996; Purrington & Bergelson, 1997, respectively). Roux et al (2004) also found a trade-off (37% reduction) in the allocation of resources to reproduction in segregating *A. thaliana* mutants possessing the same Pro-197-Ser resistance allele. This reduction in seed production has a drastic effect on plant fitness as shown by a significant decline in the frequency of the Ser-197 resistance

allele over time in the absence of herbicide selection (Roux *et al.*, 2006). This fitness cost has been shown to be greater in magnitude in nutrient limited environments, probably as a consequence of a higher N demand driven by the effects of reduced feedback inhibition and promoted higher AHAS activity and amino acid biosynthesis (Bergelson, 1994; Purrington & Bergelson, 1997; Purrington & Bergelson, 1999). However, the amino acid substitution Ser-653-Asn, which endows resistance to the AHAS-inhibiting herbicide, imazapyr, is not associated with impaired plant growth or seed germination rate in mutant *A. thaliana* (Roux et al., 2004).

Point mutations endowing resistance to auxin herbicides have been obtained through seed mutagenesis (EMS) in *A. thaliana* and evaluated for fitness costs. The Gly-459-Asp mutation in the AUX1 gene has no adverse effects on plant fitness despite changes in root morphology (Maher & Martindale, 1980; Roux *et al.*, 2004; Roux *et al.*, 2005a; Roux & Reboud, 2005). On the contrary, the AXR1 (Cys-154-Tyr) and AXR2 (Pro-87-Ser) gene mutations express a severe fitness cost. Both resistance alleles have been shown to have pleiotropic effects on leaf morphology, hypocotyl length and plant height (Lincoln *et al.*, 1990; Timpte *et al.*, 1994; Timpte *et al.*, 1995), resulting in impaired plant resource acquisition and extensive resistance costs of 78% and 89% at the reproductive stage (Roux *et al.*, 2004; Roux & Reboud, 2005).

Fitness costs have been also evaluated in three discrete laboratory originated mutations endowing resistance to isoxaben (cellulose biosynthesis inhibitor) in *A. thaliana* (Roux *et al.*, 2004). Whereas the Thr-942-Ile amino acid substitution in the cellulose synthase catalytic isoform CesA3 (Scheible *et al.*, 2001) resulted in a 43% reduction in reproductive biomass, mutations in the CesA3 (Gly-998-Asp) and

CesA6 (Arg-1064-Trp) alleles (Desprez *et al.*, 2002) did not incur fitness costs (Roux *et al.*, 2004; Roux *et al.*, 2005a). It is noteworthy that this second mutation of the CesA3 gene is located only 56 amino acid positions upstream of the mutation that incurred a 43.2% reproductive cost.

Measuring fitness costs in *A. thaliana* presents a number of opportunities that would not otherwise be available in less easily manipulated weed species. Nevertheless, some caution should be exercised in the interpretation of these results. Laboratory-derived mutants, by their nature, have not been selected in the field and therefore mutations with severe fitness costs, as seen for AXR1 and AXR2 above, may have little relevance for understanding field-evolved resistance as these mutants could not and have not evolved in the field. EMS-mutagenesis will create a range of other potentially deleterious mutations which will impact on fitness and despite crossing programs to attempt to disrupt linkage between resistance-endowing and other mutations, it is difficult to unequivocally attribute costs to the resistance allele. Transgenic approaches may be influenced by positional effects so that fitness costs are dependent on where in the genome the transgene is inserted (Purrington & Bergelson, 1999). Finally, in field-evolved populations, there may have been some compensation of the cost of resistance during selection (see Discussion) and this cannot occur in laboratory-derived mutants and may result in over-estimation of resistance costs.

VII. Fitness cost associated with herbicide resistance endowed by enhanced rates of herbicide metabolism catalysed by P450 enzymes

Cytochrome P450s are a large class of proteins involved in many biosynthetic functions in plants (reviewed in Schuler & Werck-Reichhart, 2003). In addition to

their essential role in metabolism, plant P450 enzymes are paramount in detoxification pathways and can be responsible for herbicide detoxification (Werck-Reichhart *et al.*, 2000). The current understanding of P450-mediated herbicide metabolism suggests that there are multiple P450 isoforms capable of metabolizing a specific herbicide, as well as P450 isoforms that may have broad herbicide specificity (Preston *et al.*, 1996; Werck-Reichhart *et al.*, 2000; Siminszky, 2006). However, molecular determination of the specifics of P450 involvement in herbicide metabolism remains to be identified.

Many studies have identified *L. rigidum* populations that have P450 endowed resistance to ACCase, AHAS, PSII and other herbicide chemistries (Christopher *et al.*, 1992; Burnet *et al.*, 1994; McAlister *et al.*, 1995; Preston *et al.*, 1996). We have shown that individual *L. rigidum* plants with resistance to specific AHAS and ACCase herbicides endowed by cytochrome P450-based enhanced herbicide metabolism exhibit a nearly 20% reduction in vegetative growth (Vila-Aiub *et al.*, 2005a). This reduction in resource acquisition corresponded to a reduced relative plant growth rate (RGR) resulting from lower net assimilation rates (NAR) (Vila-Aiub *et al.*, 2005a). P450-based resistant individuals showed a significantly diminished ability to grow under competition with wheat, when compared to herbicide susceptible plants. This ecological cost was expressed at both the vegetative (30%) and reproductive (23%) stages (Vila-Aiub *et al.*, 2009).

The value of studies examining the cost of P450 based resistance is evident to validate wider ecological assumptions such as the trade-off between plant growth and defence (Herms & Mattson, 1992; Vila-Aiub *et al.*, 2009). However, no other studies have attempted to assess fitness costs associated with P450 enhanced herbicide metabolism and therefore there is limited appreciation of the fitness

consequences of metabolism-based herbicide resistance. Given the importance of P450 based resistance and that plants must invest resources to produce P450 enzymes much more work is required in this area.

VIII. Fitness cost associated with glyphosate resistance

Glyphosate is the world's most widely used and important herbicide (Duke & Powles, 2008). The widespread adoption of glyphosate-resistance crops in North and South America has resulted in many populations of glyphosate resistant weeds (reviewed by Powles, 2008). In many weed populations, resistance to glyphosate correlates with reduced rates of glyphosate translocation to active meristematic root and shoot tissues (Powles & Preston, 2006; Preston & Wakelin, 2008; Shaner, 2009). *Lolium rigidum* individuals with this resistance mechanism exhibited no reduction in vegetative growth under resource competition with wheat when compared to the susceptible genotype (Pedersen *et al.*, 2007). The same study reports that glyphosate resistant plants produce fewer but larger seeds under very low competition intensity from wheat (Pedersen *et al.*, 2007). The observed reduction in the number of the progeny when competing with wheat may explain the drastic decline in the resistance phenotypic frequency from 45% to 11% after three years of relaxed glyphosate selection (Preston & Wakelin, 2008).

Ipomoea purpurea has been shown to exhibit high levels of genetic variation associated with glyphosate tolerance (Baucom & Mauricio, 2004; Baucom & Mauricio, 2008). Interestingly, a negative correlation shows that the most glyphosate tolerant individuals show the highest cost in fitness (Baucom & Mauricio, 2004).

Glyphosate resistance may also be endowed by a mutation in the target gene (EPSP synthase) (reviewed by Powles & Preston, 2006; Powles & Yu, 2010). Thus

far, there are no published studies of the effect of EPSP synthase gene mutations on fitness performance of resistant plants. Given the importance of glyphosate in world agriculture and the number of weed species displaying glyphosate resistance it is crucial that studies be conducted on the impact on fitness of resistance endowing EPSP synthase gene mutations.

IX. Discussion

1. Towards improved protocols for the estimation of herbicide resistance fitness costs.

The large body of research aimed at detecting and quantifying herbicide resistance fitness costs indicates recognition of the importance of this knowledge for understanding and predicting the dynamics of resistance evolution and management. Ultimately, resistance may be an inevitable consequence of herbicide use, yet fitness costs can considerably slow the evolution of resistance and these costs and their impacts on plant life histories can be manipulated to mitigate risks of resistance. Notwithstanding this, the literature has many studies that have misunderstood, mismeasured or misinterpreted costs of resistance. Bergelson and Purrington (1996) highlighted that very few studies examining the fitness cost of herbicide resistance controlled or minimised the differences in genetic background between the herbicide resistant and susceptible plants under study. Unfortunately, this review some 14 years later, establishes that researchers conducting fitness studies often continue to ignore the importance of genetic background in fitness studies.

In section II, we presented five criteria that should inform the design and interpretation of future studies to determine costs of herbicide resistance alleles. It will not always be possible or practical to fulfil all of these. However, studies which

do not control for genetic background and have little knowledge of the mechanism of resistance will be of limited value to ascribe the origin of fitness costs to particular resistance genes and mutations. Beyond these basic requirements, studies should estimate fitness costs in realistic field conditions under which resistance evolved and consider fitness costs and implications throughout the plant life cycle. Fortunately, in recent years a relatively small number of unequivocal fitness cost studies have been conducted. We believe that this trend, if it persists, will allow a systematic evaluation of fitness costs which ultimately will be of potential significance for the management of resistance and for providing answers to fundamental questions in plant evolutionary ecology.

2. Future advances in understanding costs of herbicide resistance.

Routine consideration of the five criteria in Section II will considerably advance studies of herbicide resistance costs. In future, as molecular tools are developed for weed species, further hypotheses relating to the 'cost of adaptation' may be addressed, both for their benefit in terms of resistance management and for their ability to answer more fundamental questions in plant adaptation. Some of these future research areas are outlined below.

The importance of a homogenised genetic background for measuring fitness costs has been argued. In selfing species, resistance alleles will arise in a variety of genetic backgrounds and, for outcrossing species, the genetic background in which the resistance allele occurs may vary between individuals within a single population. It is likely that the magnitude and expression of fitness costs will vary between different genetic backgrounds, presenting the possibility that fitness costs will vary between populations and between individuals (Paris *et al.*, 2008). These effects

should be investigated. Furthermore, in outcrossing species, there may be selection so that resistance alleles are preferentially expressed in backgrounds where the cost is lowest. Evolutionary forces will select for genetic backgrounds in which the costs are minimised through i) the acquisition of modifiers (i.e. new alleles at other loci) which compensate and moderate pleiotropic costs (Paris *et al.*, 2008), ii) replacement of alleles which involves the substitution of the costly allele by a less costly one at the locus involved in resistance and iii) increased resistance to biotic stresses (parasitism) (Agnew *et al.*, 2004). These amelioration processes will lead to a reduction in the magnitude of fitness costs over plant generations and represent one form of compensatory evolution such that costs of resistance evolve over time (resistance co-adaptation). The potential for resistance co-adaptation is poorly understood for herbicide resistance, though it has been demonstrated for insecticide resistance (McKenzie & Clarke, 1988; Bourguet *et al.*, 2004) and antibiotic resistance (Maisnier-Patin & Andersson, 2004; Paulander *et al.*, 2007). These processes create the opportunity for co-adaptation and integration of the new resistance allele into the genome (Fisher, 1928; Cohan *et al.*, 1994; Guillemaud *et al.*, 1998)

Only a few studies have considered the dominance of the herbicide resistance cost. In the field, new herbicide resistance alleles which arise by spontaneous mutation will occur as heterozygotes and therefore it is most important to understand the fitness consequences of resistance alleles in the heterozygous state. If the cost of resistance is recessive (i.e. only evident in homozygous individuals), then the fitness cost will have little impact on the dynamics of resistance in the early stages of selection where most individuals are heterozygous. Using EMS-mutagenised *A.*

thaliana, Roux et al. (2004) demonstrated recessive, dominant and over-dominant herbicide resistance fitness costs.

Herbicide resistance endowing mutations are likely to occur as independent events and this may result in the accumulation of diverse herbicide resistance alleles within single individuals under herbicide selection (Hall *et al.*, 1994; Tardif & Powles, 1994; Preston *et al.*, 1996). From a fitness cost viewpoint, an interesting question is to consider the epistatic effects (e.g. multiplicative, additive, synergistic, and compensatory) of two or more different resistance alleles on fitness cost when there are multiple resistance alleles within an individual. A study with the model plant *A. thaliana* has revealed multiplicative epistatic effects on several fitness traits in double and triple mutants expressing the ALS (Pro-197-Ser), 2,4-D (Cys-154-Tyr) and isoxaben (Thr-942-Ile) resistance alleles (Roux *et al.*, 2005b). This result predicts that the frequency decline of multiple resistance alleles in an herbicide-free environment would be faster than when epistatic interactions among resistance alleles are not evident.

3. The evidence for pleiotropic effects of herbicide resistance alleles.

There is unquestionable evidence, as reviewed here, that some herbicide resistance alleles are associated with fitness costs. Observed costs are associated with target site resistance (resistance endowing amino acid substitutions in proteins involved in amino acid (Section III), fatty acid (Section II), auxin (Section V) and cellulose (Section VI) biosynthesis) as well as non target site resistance due to enhanced rates of herbicide metabolism (Section VII) and transport (Section VIII). However, resistance costs are not universal and thus they must be assessed on a case-by-case basis. Fitness costs associated with target-site resistance generally result from a

compromise in normal enzyme function whereas costs associated with metabolic resistance are thought to arise from allocation of resources to defence rather than growth (Coley *et al.*, 1985; Herms & Mattson, 1994; Strauss *et al.*, 2002; Vila-Aiub *et al.*, 2009).

4. Evolution of herbicide resistance alleles and their fitness costs

Theoretical models predict that resistance alleles with the largest fitness costs are least likely to be fixed (Gillespie, 1975). This prediction appears to be confirmed for some ACCase gene mutations which show a negative correlation between the magnitude of fitness costs and their frequency in agroecosystems. An extensive survey in France has revealed that the Asp-2078-Gly ACCase mutation is the least frequent resistance allele in *A. myosuroides* (Délye *et al.*, 2007). This mutation has been shown to be associated with impaired plant performance at the enzyme and whole plant level causing the mutation to be negatively selected in the absence of ACCase herbicide selection pressure. On the contrary, the Ile-1781-Leu ACCase mutation in *A. myosuroides* has no detectable pleiotropic effect on plant fitness (Menchari *et al.*, 2008) and has been found to be the most common resistance allele in field crops (Délye *et al.*, 2007). Similar results have been found for both the Asp-2078-Gly and Leu-1781 resistance alleles have been found in *L. rigidum* (Vila-Aiub *et al.*, 2005a; Owen *et al.*, 2007; Yu *et al.*, 2007a). The presence of the Ile-1781-Leu ACCase mutation in more weed species than any other ACCase allele as well as its fixation in naturally resistant species is a good indication of its lack of fitness cost (Délye, 2005; Délye & Michel, 2005; Liu *et al.*, 2007; Yu *et al.*, 2007a).

The same analogy may be applied for the Pro-197-Ser and Trp-574-Leu AHAS mutations which have been reported to exhibit respectively moderate and

severe pleiotropic effects on *A. thaliana* and *A. powellii* fitness (Bergelson *et al.*, 1996; Roux *et al.*, 2004; Roux *et al.*, 2005a; Tardif *et al.*, 2006). Whereas the former is reported as the most common resistance allele in several *Raphanus raphanistrum* populations from Australia the latter has not to date evolved within this species (Friesen and Powles, unpublished).

XII. Conclusion

Fitness costs associated with herbicide resistance alelless are evident but not universal among plant species. Their expression and magnitude are strongly influenced by the abiotic, biotic and genetic environment. Future research efforts should compartmentalise the effects of these 'environments' by assessing the pleiotropic effects of resistance alleles on plant fitness with proper control of genetic background and, ideally, where the molecular basis of resistance is known. These studies should integrate molecular, physiological and ecological techniques to measure fitness at all life history stages and in a variety of biotic and abiotic environments. Field experiments that record changes in resistance allele frequencies in the absence of herbicide selection should complement these studies. Only then will it be possible to systematically elucidate whether and under which environmental conditions herbicide resistance alleles express fitness costs.

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Table 1A

AHAS mutations in evolved field selected weed species in which fitness studies have been conducted

Amino acid residue		Cross-resistance to AHAS inhibiting herbicide families	Weed species	Fitness cost	Reference
Wild type	Resistant				
Pro-197	His	Sulfonylurea	*Lactuca serriola*	Yes	Alcocer-Ruthling et al. (1992a, b)
Trp-574	Leu	Imidazolinone Sulfonylurea Pyrimidinylthiobenzoates Triazolopyrimidines Sulfonylaminocarbonyl-triazolinone	*Amaranthus powellii*	Yes	Tardif et al (2006)

Table 1B

Known AHAS mutations in evolved field selected weed species remaining to be investigated for fitness costs

Amino acid residue		Cross-resistance to AHAS inhibiting herbicide families	Weed species
Wild type	Resistant		
Ala-122	Thr	Imidazolinone	*Amaranthus hybridus, A. powellii, A. retroflexus, Solanum ptycanthum, Xanthium strumarium*
Pro-197	Ala		Several weed species
	Arg		*Kochia scoparia, Lolium rigidum*
	Gln		*Kochia scoparia, Lindernia spp, Lolium rigidum*
	His		*Raphanus raphanistrum*
	Ile	Sulfonylurea	*Sisymbrium orientale*
	Leu		*Many weed species*
	Lys		*K. scoparia*
	Met		*K. scoparia*
	Ser		*Many weed species*
	Thr		*Many weed species*
	Trp		*K. scoparia*
Ala-205	Val	Imidazolinone Sulfonylurea	*A. retroflexus, Helianthus annus, Solanum ptycanthum, Xanthium strumarium*
Asp-376	Glu	Imidazolinone Sulfonylurea Pyrimidinylthiobenzoates Triazolopyrimidines Sulfonylaminocarbonyl-triazolinone	*A. hybridus*
Trp-574	Arg	Sulfonylurea	*K. scoparia*
	Leu		*Lolium rigidum*
Ser-653	Thr	Imidazolinone	*A. powelli, A. retroflexus, A. rudis*
	Asn Ile	Imidazolinone	*A. hybridus, A.rudis, Setaria viridis*
Gly-654	Glu	Imidazolinone	*Oryza sativa*
	Asp		*S. viridis*

Table 2A

ACCase mutations in evolved field selected weed species in which fitness studies have been conducted

Amino acid residue		Cross-resistance to ACCase inhibiting herbicide families	Weed species	Fitness cost	Reference
Wild type	Resistant				
Ile-1781	Leu	Aryloxyphenoxypropionates Cyclohexanediones	*Alopecurus myosuroides*	No	Menchari et al (2008)
			Lolium rigidum	No	Vila-Aiub et al (2005a, b)
Ile-2041	Asn	Aryloxyphenoxypropionates	*Alopecurus myosuroides*	No	Menchari et al (2008)
Asp-2078	Gly	Aryloxyphenoxypropionates Cyclohexanediones	*Alopecurus myosuroides*	Yes	Menchari et al (2008)
			Lolium rigidum	Yes	Vila-Aiub and Powles (unpublished)
Cys-2088	Arg	Aryloxyphenoxypropionates Cyclohexanediones	*Lolium rigidum*	Yes	Vila-Aiub and Powles (unpublished)

Table 2B

Known ACCase mutations in evolved field selected weed species remaining to be investigated for fitness costs

Amino acid residue		Cross-resistance to ACCase inhibiting herbicide families	Weed species
Wild type	Resistant		
Trp-1999	Cys	Aryloxyphenoxypropionates (Fenoxaprop only)	*Avena sterilis*
Trp-2027	Cys	Aryloxyphenoxypropionates	*Alopecurus myosuroides* *A. sterilis, A. fatua* *Lolium rigidum* *Phalaris minor*
Ile-2041	Val	Aryloxyphenoxypropionates	*L. rigidum*
Gly-2096	Ala	Aryloxyphenoxypropionates	*A. myosuroides* *P. paradoxa*

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