Review

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Deciphering the evolution of herbicide resistance in weeds

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Resistance to herbicides in arable weeds is increasing rapidly worldwide and threatening global food security. Resistance has now been reported to all major herbicide modes of action despite the development of resistance management strategies in the 1990s. We review here recent advances in understanding the genetic bases and evolutionary drivers of herbicide resistance that highlight the complex nature of selection for this adaptive trait. Whereas early studied cases of resistance were highly herbicide-specific and largely under monogenic control, cases of greatest concern today generally involve resistance to multiple modes of action, are under polygenic control, and are derived from pre-existing stress response pathways. Although 'omics' approaches should enable unraveling the genetic bases of complex resistances, the appearance, selection, and spread of herbicide resistance in weed populations can only be fully elucidated by focusing on evolutionary dynamics and implementing integrative modeling efforts.

Herbicide-resistant weeds: a growing threat to food security

Arable weeds (see Glossary) have been the major biotic cause of crop yield losses since the origins of agriculture. Weeds result in 34% loss of crop yield, on average, worldwide [1]. In the USA alone, the annual cost of crop losses due to weeds is greater than 26 billion US$ [2]. Weeds are thus a major threat to food security. Early in agriculture, weed control was labor-intensive and only moderately effective until the first herbicides were marketed in the late 1940s [1]. Herbicides are by far the most effective weed control tools ever developed, killing 90 to >99% of the weeds targeted (e.g., [3]). Non-chemical weeding can achieve similar efficacies only by combining multiple methods, each generally far more labor-intensive than herbicide application [4]. Consequently, the arable surface treated and range of weed species targeted by herbicides increased rapidly worldwide after their development [1], and a diversity of herbicides are currently used by growers (Figure 1 and Table 1).

This golden age of herbicides was quickly cut short, however, by the detection of the first herbicide-resistant weeds in 1957 [5]. Today, herbicide resistance has been reported in 217 weed species in more than 670 000 fields worldwide (a conservative estimate). Moreover, the number of cases collated at http://www.weedscience.org is continuously rising. Resistance has been reported to all major known herbicide modes of action (see: http://www.weedscience.org), and no new mode of action has been marketed since 1991 [6].

Herbicide resistance is now widely recognized as the result of the adaptive evolution of weed populations to the intense selection pressure exerted by herbicides [7,8]. The least herbicide-sensitive individuals have a selective advantage in weed populations repeatedly treated

Glossary

Allogamy/autogamy: cross-fertilization/self-fertilization in plants. The degree of allogamy and autogamy varies among and within species; mating systems intermediate between complete autogamy and complete allogamy are termed mixed-mating systems.
Arable weed: plant growing unwanted in a cultivated field. Weeds include numerous, diverse, mostly annual short-lived plant species that thrive in highly disturbed agricultural ecosystems.
Constitutive (response/pathway): a response/pathway that is continually activated, as opposed to a response/pathway that is only activated in response to an environmental signal (induced response/pathway).
Cross-resistance: resistance to different herbicides caused by one gene or one mechanism (as opposed to multiple resistance: resistance to different herbicides caused by different genes or different mechanisms).
Effective population size: the size of an ideal population that experiences the same amount of genetic drift as the observed population. In this ideal population, all individuals have an equal probability of contributing to the next generation via reproduction. The effective population size is typically smaller than the actual number of individuals in a population (termed the census population size).
Epigenetic process: process altering gene regulation that does not involve changes in the DNA sequence (e.g., DNA methylation, histone protein modifications). These changes can persist through cell divisions and be transmitted to progeny.
Fitness cost: adaptation to a selective pressure endows a fitness cost when it results in a decrease in the ability of an organism to survive and/or reproduce in the absence of the selective pressure. Fitness cost can result from antagonistic pleiotropy.
Herbicide: synthetic organic molecule used to kill weeds. Herbicides act by lethally inhibiting the activity of proteins crucial for weed physiological processes.
Pleiotropy, antagonistic pleiotropy: phenomenon where one gene influences multiple phenotypic traits in an organism. Antagonistic pleiotropy occurs when selective pressure upon a given trait changes the mean value of a correlated trait that has a negative effect on fitness.
Selective sweep: the rapid increase in frequency of a beneficial allele due to selection that also reduces genetic variation at linked loci.
Soil seed bank: the natural stock of seeds in the soil, which are usually dormant but viable. The soil seed bank is generally the major source of weeds that infest crops.
Standing genetic variation: the presence of multiple alleles at a locus in a population. Genetic variation that is neutral or nearly neutral (i.e., that results from the opposing forces of mutation and genetic drift) may become beneficial after an environmental change.

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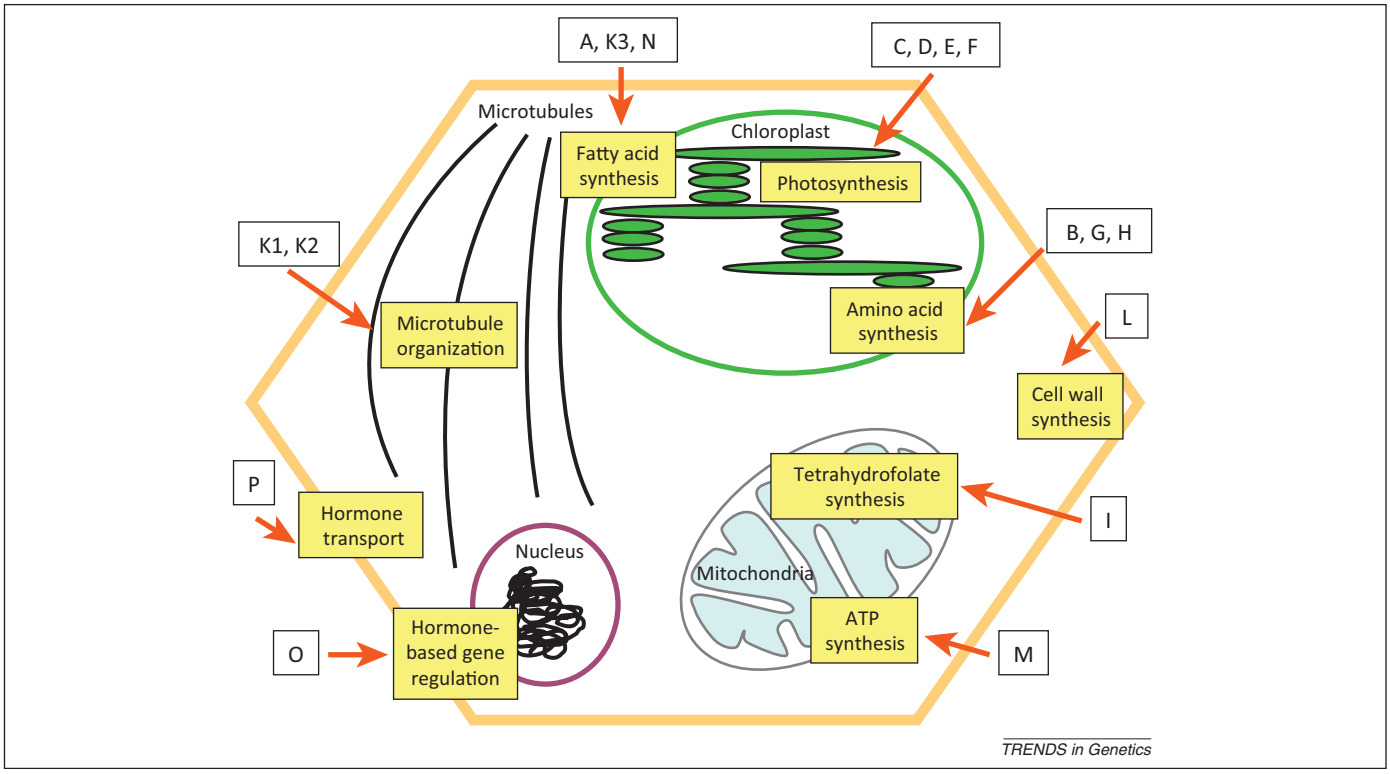

Figure 1. Cellular targets of herbicide action and herbicide classification by mode of action according to the Herbicide Resistance Action Committee (HRAC). Herbicides target only a few proteins or processes among the tremendous range present in plants.

Table 1. Herbicide modes of action

HRAC group[a]	Herbicide mode of action	Gene encoding target protein	Pathway or process targeted
A	Inhibition of acetyl-CoA carboxylase (ACCase)	Nuclear	*Fatty acid biosynthesis*
B	Inhibition of acetohydroxyacid synthase (AHAS, ALS)	Nuclear	*Amino acid biosynthesis* (Leu, Ile, Val)
C	Inhibition of photosystem II protein D1 (psbA)	Chloroplastic	*Photosynthesis* (electron transfer)
D	Diversion of the electrons transferred by the photosystem I ferredoxin (Fd)	Chloroplastic	*Photosynthesis* (electron transfer)
E	Inhibition of protoporphyrinogen oxidase (PPO)	Nuclear	*Photosynthesis* (heme biosynthesis for chlorophyll)
F	Inhibition of phytoene desaturase (PDS) or 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) or of an unknown protein	Nuclear	*Photosynthesis* (carotenoid biosynthesis)
G	Inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase)	Nuclear	*Amino acid biosynthesis* (Phe, Trp, Tyr)
H	Inhibition of glutamine synthase	Nuclear	*Amino acid biosynthesis* (Gln)
I	Inhibition of dihydropteroate synthase	Nuclear	Tetrahydrofolate biosynthesis
K1, K2	Enhancement of tubulin depolymerization	Nuclear	Microtubule polymerization
K3	Inhibition of fatty acid synthase (FAS)	Nuclear	*Fatty acid biosynthesis*
L	Inhibition of cellulose-synthase	Nuclear	Cell wall biosynthesis
M	Uncoupling of oxidative phosphorylation	-	ATP biosynthesis
N	Inhibition of fatty acid elongase	Nuclear	*Fatty acid biosynthesis*
O	Stimulation of transport inhibitor response protein 1 (TIR1)	Nuclear	Regulation of auxin-responsive genes
P	Inhibition of auxin transport	Unknown	Long-range hormone signaling
Z	Unknown		

[a]The 18 groups of herbicides classified by mode of action according to the global Herbicide Resistance Action Committee (HRAC; http://www.hracglobal.com). The three most widely used groups are indicated in bold. Pathways or processes targeted by several herbicide groups are indicated in italic text.

with herbicide and thus increase in frequency until populations shift towards a predominance of herbicide-resistant individuals. Resistant weeds can survive herbicide application via a variety of mechanisms [9–11] that can be divided into two broad categories. Target-site resistance (TSR) mechanisms include increased expression of the target protein or structural changes to the herbicide binding site (Figure 2). Non-target-site resistance (NTSR) mechanisms include any other mechanism (see Figure 2 for mechanisms currently reported).

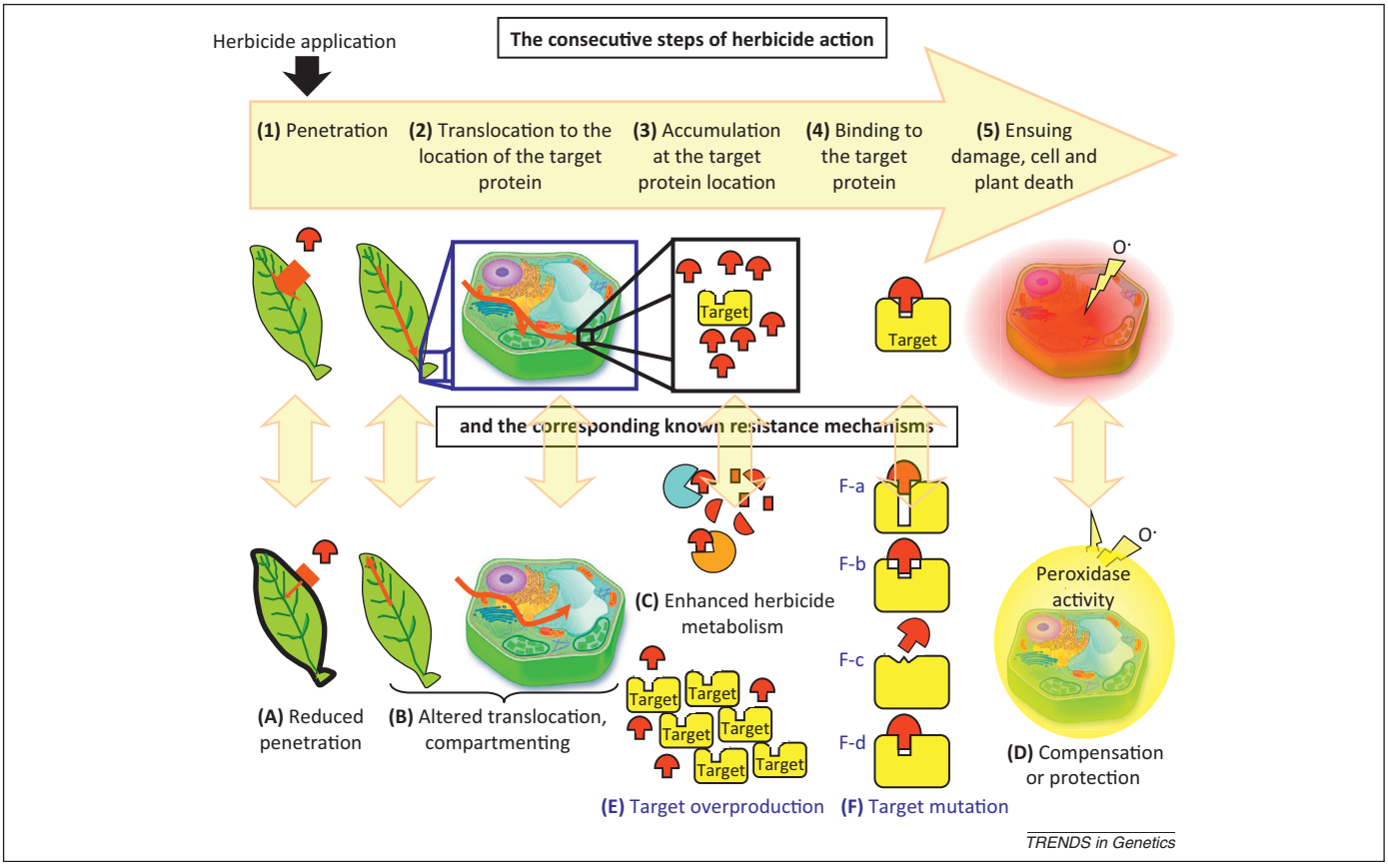

Figure 2. The action of herbicides (top) following their application and the resistance mechanisms identified in weeds that correspond to each action step (bottom). After application, (1) herbicide molecules penetrate the plant, (2) are translocated to the location of the target protein (here, the chloroplast of meristem cells), (3) accumulate at the location of the target protein, and (4) bind to the target protein, thereby (5) disrupting biosynthesis pathways or vital cell structures, and/or generating cytotoxic molecules (e.g., active oxygen that damage cells and ultimately causes plant death). Multiple mechanisms of resistance interfering with the herbicide action steps have evolved in weeds. Non-target-site resistance (NTSR) mechanisms include **(A)** reduction in herbicide penetration due to alterations in cuticle properties and/or plant habit, **(B)** altered translocation of the herbicide away from the target protein, **(C)** enhanced degradation (metabolism) of the herbicide, or **(D)** enhanced neutralization of cytotoxic molecules generated by herbicide action (illustrated: neutralization of active oxygen by peroxidases). Target-site resistance (TSR) mechanisms include **(E)** regulatory mutations causing target protein overproduction that compensates for the herbicide inhibitory action, and/or **(F)** structural mutations that modify the 3D structure and electrochemical properties of the target protein. Structural mutations can have no, moderate, or strong negative effects on the stability of herbicide binding to the target protein, which result in (F-a) no, (F-b) moderate or (F-c) marked reduction in herbicide sensitivity at the protein level, respectively; or can (F-d) increase the stability of herbicide binding to the target protein, which results in an increase in herbicide sensitivity (i.e., hypersensitivity) at the protein level.

This review focuses on the recent progress made in understanding the genetic and evolutionary mechanisms underlying herbicide resistance in weeds. Current controversies on key aspects of resistance evolution are discussed. We also highlight crucial future research directions and associated challenges.

Target-site resistance (TSR): monogenic resistance, but not so simple

The first herbicide-resistant weeds to be identified were intensively investigated in the 1980s–90s. In most cases, resistance was conferred via TSR mechanisms determined by dominant alleles at a single nuclear gene locus [9,10,12,13]. To date, nuclear monogenic control of TSR has been identified to herbicide groups A, B, K1, K2, E, and G (Figure 1 and Table 1), whereas inheritance of TSR to triazine herbicides (group C) is cytoplasmic. TSR is particularly widespread to herbicides in groups A, B, and C [9,10,12,13].

Recent advances indicate that nuclear monogenic TSR is less simple than previously thought. Although most TSR cases are indeed conferred by dominant or semi-dominant alleles (reviewed in [9,10]), recessive control of TSR has also been reported for resistance to herbicides in group K (e.g., [14]). TSR is largely endowed by changes in the 3D structure of the herbicide target protein and in the distribution of polar groups at positions crucial for the stability of herbicide binding to the protein (Figure 2) (e.g., [15–17]). Structural changes are generally due to amino-acid substitutions at one of several possible positions on the herbicide target protein. Known substitutions have recently been collated ([9,10,12,13]; see also http://www.weedscience.org/Mutations/MutationDisplayAll.aspx). As a general rule, several substitutions conferring resistance are possible at a given crucial codon: as many as 12 substitutions endowing resistance have been identified at codon 197 in acetohydroxyacid synthase, the target protein of group B herbicides ([10]; see also http://www.weedscience.org/Mutations/MutationDisplayAll.aspx). The magnitude of reduction in affinity of a herbicide for its binding site depends both on the structural change in the target protein and on the herbicide molecule. Depending on the herbicide, a given structural change in the target protein can confer high or moderate resistance (e.g., [18,19]) or, in rare instances, an increase in sensitivity to the herbicide (e.g., [14]) (Figure 2). Thus, contrary to the conclusions of

early studies, the emerging picture of TSR is not in black and white, but in shades of gray.

In fungi, TSR to fungicides is frequently due to an accumulation of several amino acid substitution(s) and/or deletion(s), sometimes combined with increased production of the target protein [20]. The situation is similar for TSR to insecticides in *Drosophila* [21]. Fungi and *Drosophila* are eukaryotic organisms with tremendous population sizes and multiple reproductive cycles per year that allow adaptive evolution at a much faster rate than is possible in plants. Interestingly, a few recent studies of TSR to herbicides have also revealed complex genetic changes in weeds, including the deletion of an entire codon [22], successive amino acid replacements resulting from two consecutive nucleotide substitutions at the same codon [23], accumulation of two amino acid substitutions at distinct codons that increased the resistance level compared to a single mutation [24], and an increase in synthesis of the target protein [25,26]. These mechanisms seem currently infrequent in weeds, possibly because they involve genetic changes with a low probability of appearance. However, the parallels with TSR to fungicides and insecticides suggest that future work into the genetics of TSR to herbicides may reveal more complex mechanisms.

Evolution of TSR is assumed to conform to the selective sweep model of adaptation (*sensu* Pritchard [27]) where a single beneficial mutation of large effect allows the initial survival of mutants and then spreads quickly because of positive selection. Numerous factors influence the evolution of TSR, including the rates of mutation and initial frequencies of resistance alleles in weed populations, their inheritance and effect on plant fitness in the presence and absence of herbicides, the pattern of mating among individuals within populations, and the extent of gene flow among populations [7]. Simple population genetic models have proven helpful to integrate the effects of these evolutionary factors in the past and to assess the efficacy of various management strategies in reducing the probability of, and time to, resistance evolution (Box 1). However, the recent rapid increase in the abundance and geographical extent of herbicide resistance is largely due to non-target-site-based resistance (NTSR) (e.g., [13,28]), the evolution of which cannot be investigated by models used to address TSR.

NTSR: a complex polygenic adaptation to herbicides that remains to be elucidated

Only recently has the importance of NTSR been fully comprehended. NTSR is now considered the predominant type of resistance to the first and third most important herbicide groups worldwide, namely groups G (glyphosate) and A (acetyl-CoA carboxylase inhibitors) [9–11,13]. NTSR is also the predominant type of resistance to the worldwide second most important herbicide group (group B: acetohydroxyacid synthase inhibitors) in grasses [9–11], and its importance in broadleaved weeds (dicotyledons) is most likely underestimated (e.g., [29]). Moreover, NTSR is the only type of resistance identified to date to herbicide groups D, N, O, and Z [10,30]. The most negative aspect of NTSR is an associated cross-resistance that remains unpredictable and is not specific to the mode of action of the selecting herbicide. Thus, NTSR mechanisms selected by a given herbicide can confer cross-resistance to herbicides with other modes of action, including those not yet marketed (e.g., [31]).

In essence, NTSR appears to be a more general adaptive response to herbicides than TSR. NTSR mechanisms are a subset of plant responses to abiotic stresses [11,32]. NTSR can be constitutive, stress-induced, or possibly partly both [33]. Constitutive NTSR has been shown to be associated with higher secondary metabolism in plants [33]. The current hypothesis for induced NTSR is that herbicide application is a stress that triggers response pathways in all weed individuals irrespective of their sensitivity to the herbicide [11]. Genetic variation in the magnitude of response among individuals results in variation in sensitivity, and this is the basis for the evolution of induced NTSR. NTSR mechanisms are expected to be diverse (Figure 2), and to differ among and within species. This is consistent with demonstrations of complex multigenic control of NTSR in grass weeds [34,35], although monogenic NTSR can exist [36]. NTSR mechanisms can also vary with the herbicide: whereas altered translocation of

Box 1. Strategies for managing the evolution of herbicide resistance

By far, the most common management strategies recommended to farmers to combat herbicide resistance are to apply mixtures, sequences (within the same growing season), or rotations (over multiple growing seasons) of herbicides with different modes of action in their fields. Such diversity in herbicide use reduces the intensity of selection for resistance to any one herbicide. However, because NTSR often confers cross-resistance to different herbicide modes of action, managing resistance as a whole not only requires using herbicides with different modes of action, but also herbicides in which different metabolic pathways are involved in their degradation, when known.

(i) *How are resistance management strategies evaluated?*

Because field experiments are difficult to implement, there is limited empirical evidence on the effectiveness of resistance management strategies under realistic agronomical conditions (but see [79]). Most often, strategies have been evaluated using simulation modeling (e.g., [80]). More recently, experiments conducted with the model organism *Chlamydomonas reinhardtii* have proved useful for assessing the probability and length of time to resistance evolution under various herbicide management strategies while allowing for large population sizes and many generations of selection [76,81]. Interestingly, the latter studies showed that herbicide rotations may not always slow the rate of resistance evolution, and may result in the evolution of generalist phenotypes resistant to a broad range of herbicide modes of action.

(ii) *Which strategy is best?*

Herbicide mixtures are more likely to delay the evolution of multiple TSR in weeds than herbicide rotations [81] because the probability of plants with multiple resistant target-sites is the product of the probabilities of mutation at each site of action, and thus is very low. Herbicide mixtures most effectively delay resistance only when the herbicides used have similar efficacies on the weed targeted. If not, resistance is likely to evolve to the herbicide exerting the stronger selection [82]. Moreover, when resistance has already evolved to one herbicide, using it in a mixture with a second herbicide is inefficient [83]. Based on these rationales, the promotion of new transgenic crops with stacked herbicide resistance traits and genes (e.g. [84]) as a tool to manage resistance has been criticized by scientists who advocate that weed management be integrated and not based solely on diversifying herbicides [85].

the herbicide is the major resistance mechanism to herbicides in groups G (glyphosate) and D, enhanced metabolism seems the major resistance mechanism to herbicides in groups A and B [9,10]. Protection against oxidative damages subsequent to herbicide action may also play an important part in NTSR [33,37,38].

The regulation of NTSR pathways involved in herbicide sensing and herbicide stress-response triggering remain unknown. Several families of proteins have been identified in resistant weeds that play a part in herbicide compartmentalization (transporter proteins), degradation (cytochrome P450s, glutathione-*S*-transferases, glycosyltransferases, esterases, hydrolases), and compensation (oxidases, peroxidases) (reviewed in [9,11,32]). NTSR may be conferred by modified regulation(s) of genes encoding such proteins and/or amino acid changes conferring an increased capacity for herbicide degradation [11].

Studies of the genetic bases of NTSR are lacking. Only the genes encoding two glutathione-*S*-transferases and one esterase have been identified [33,37–39]. Research addressing NTSR in weeds has long been hampered by the absence of 'omics' resources for the vast majority of weed species. Increasing accessibility of genomics and transcriptomics supported by next-generation sequencing technologies should rapidly enable the identification of genes governing NTSR. 'Omics'-based approaches have recently been reviewed for this purpose [11,32]. Transcriptomes are already available for 15 broadleaved (dicotyledonous) weed species [40–43].

The evolutionary rescue of weed populations

Preliminary findings suggest that NTSR originates from complex abiotic stress-response pathways pre-existing within plant species (reviewed in [11]). Alleles with minor effects on resistance may thus be present at many gene loci in unselected, herbicide-susceptible populations. Thus, whereas TSR evolves from the selection of mutations at a single gene locus, NTSR evolution is likely to result from selection acting on standing genetic variation at multiple gene loci. Across any given field, herbicide applications are not homogeneous. Thus, some weed individuals may receive reduced doses and survive if they carry some NTSR alleles. Further NTSR evolution probably requires several generations of sexual recombination within a population until individuals accumulate enough alleles to confer a resistance level enabling survival to the full dose of herbicide. Recurrent selection experiments at reduced herbicide rates, which increase plant survival, convincingly demonstrated the buildup of polygenic resistance to herbicides in groups A, G, and K3 [44–49]. Accumulation of genetic factors via recombination is only possible in allogamous (cross-fertilizing) species. Herbicide selection experiments conducted with the highly autogamous (self-fertilizing) species *Arabidopsis thaliana* were unsuccessful in increasing resistance levels despite initial genetic variation for herbicide sensitivity [50].

The standing genetic variation for NTSR in weed populations never previously exposed to herbicides remains largely unknown. Approximately 0.4% of individuals, on average, survived field doses of diclofop-methyl (group A) [51] and glyphosate [52] within naive populations of *Lolium rigidum* and *Amaranthus rudis*, respectively. Because NTSR can confer cross-resistance to herbicides with different modes of action, selection of NTSR by one herbicide applied over several generations may considerably facilitate the evolution of NTSR to another herbicide [49]. Thus, rotations or mixtures of herbicides that differ in mode of action may provide continuous strong selection for NTSR and, contrary to the intent of the commonly prescribed management strategies (Box 1), increase the

Box 2. Evolutionary rescue (ER): a new concept for understanding resistance evolution

The concept of ER has recently gained much importance because it contributes to a new understanding of processes such as adaptation to environmental change and the evolution of resistance to pesticides and drugs [53]. In contrast to conventional population genetic models, ER considers genetically determined variation in absolute, rather than relative, fitness and its effect on the eco-evolutionary dynamics of populations. Absolute fitness is the potential for individuals of a given genotype to survive and reproduce in the face of selection. A genotype with an absolute fitness >1 increases in number in the population. Following an abrupt environmental change, such as the application of a herbicide onto a susceptible weed, most genotypes in a population are maladapted with absolute fitness <1, which results in a major reduction in population size, in other words a demographic decline. ER occurs when a few pre-existing adapted genotypes, or an adapted genotype arising by *de novo* mutation, reproduce at a sufficient rate to avoid population extinction. This results in a U-shaped curve for population size over time (Figure I).

Several factors condition ER, among which the initial population size, the genetic architecture and standing genetic variation for the adaptive trait, and the mutation supply rate, are likely to be important in determining the ER of populations to pesticides via resistance evolution. Larger initial population sizes increase the likelihood of ER because the probability that a rescue variant is present or appears in the population is higher, and there is a longer waiting time for this variant to appear and spread before a population becomes extinct [53]. Regarding genetic architecture and variation, ER depends on the absolute copy number of a beneficial allele, which makes ER less probable when adaptation relies on new mutations at one or a few loci [86]. On the other hand, when standing variation at many loci of small effect contribute to fitness, the probability of ER decreases because selection per locus is increasingly weakened with more loci. Notably, the probability of ER is higher when both major and minor genes contribute to adaptation [87], as is often the case for herbicide resistance.

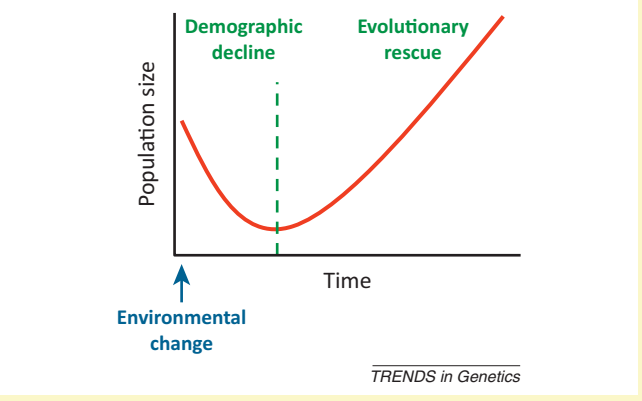

Figure I. The collapse and recovery of a population during evolutionary rescue.

probability of resistance evolution rather than reduce its likelihood.

TSR and NTSR differ in their genetic determinism and modes of evolution. Even so, they evolve together under the selection pressure exerted by herbicides: both can occur within the same species, the same population, and even the same individual (e.g., [28,31]). Either TSR or NTSR can predominate according to the selecting herbicide and the species considered. Although NTSR seems predominant in grasses, there is growing evidence that its importance in broadleaved weeds has been underestimated [29]. Recent increases in populations and species with both TSR and NTSR clearly indicate that we now need an integrative conceptual framework to explain the evolution of herbicide resistance as a whole. 'Evolutionary rescue' (*sensu* Gonzalez [53]) may well fulfill this need. This emerging topic in evolutionary biology focuses on populations facing a sharp environmental change that can potentially drive them to extinction. Evolutionary rescue occurs when genetic adaptation is rapid enough to allow demographic recovery (Box 2). Recently, a simulation model integrating the population dynamics of a weed with the evolution of a resistance trait with an explicit genetic basis [54] challenged the current view in the literature that TSR evolves more rapidly than NTSR by demonstrating that polygenic resistance generally evolves first, provided that minor resistance genes initially occur in higher frequencies in a weed population than a major gene.

Multiple evolutionary origins of resistance

Several recent studies have surveyed numerous field populations of weeds for herbicide resistance across broad geographical ranges [28,55–58]. In all cases, high intra- and interpopulation variability for resistance was observed. Moreover, when genetic data were used to assess the evolutionary origins of resistance, multiple independent origins were the rule [28,58,59]. The independent evolution of resistance in multiple populations over short time-scales requires very large population sizes, such that virtually all beneficial mutations have a non-negligible

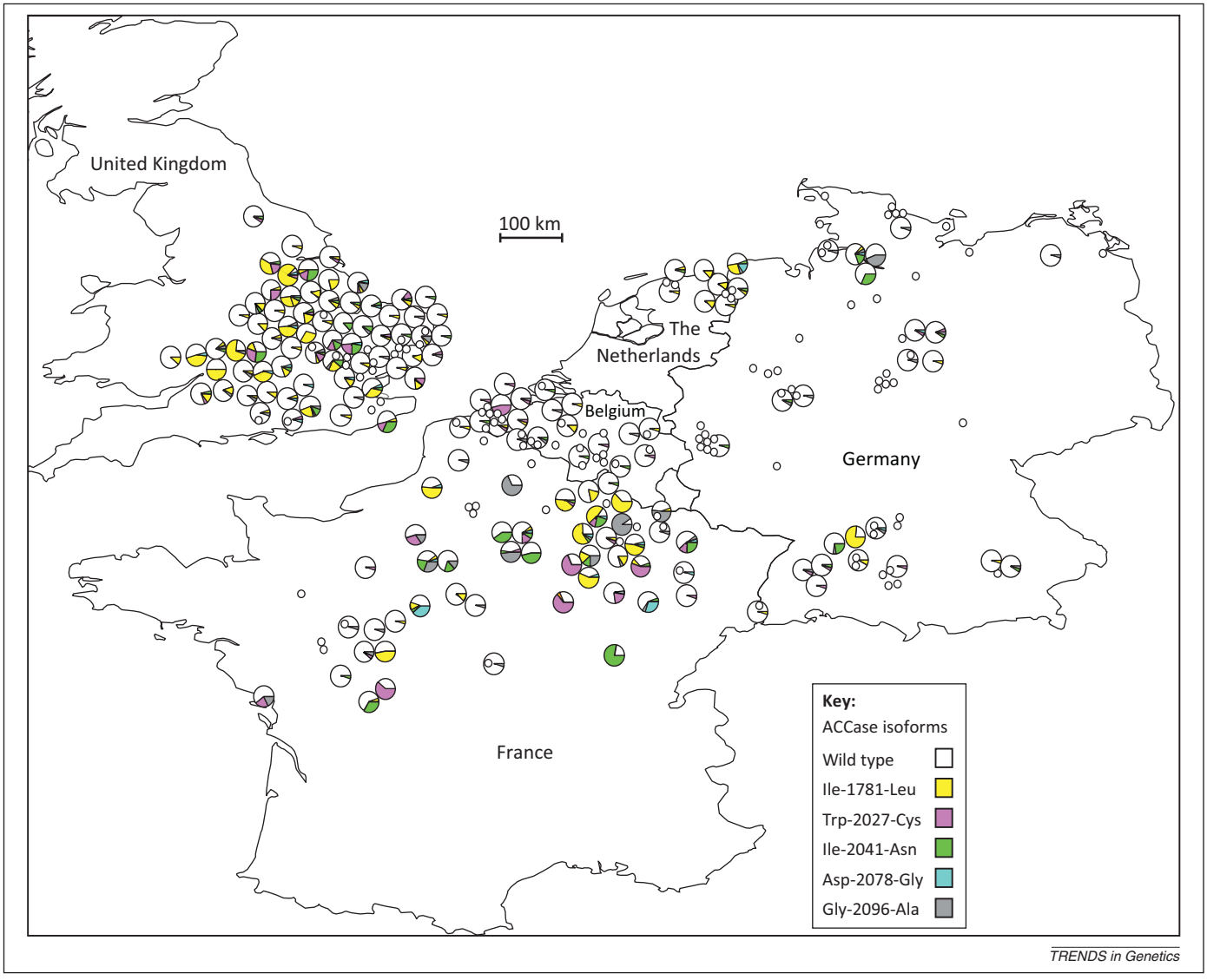

Figure 3. Geographical distribution of mutant herbicide-resistant variants of the target protein acetyl-CoA carboxylase (ACCase) of group A herbicides (Figure 1) among 297 populations of the grass weed *Alopecurus myosuroides* in Europe. Pie charts show the respective frequencies of the mutant acetyl-CoA carboxylase variants detected in populations. Populations where no mutant variant was detected are shown as small, white circles. Sequence data demonstrated multiple, independent appearances of each mutant variant in different populations, and even within the same population [28].

probability of being already present when selection takes place [60]. In weeds, high reproductive capacity and occurrence of soil seed banks can contribute to large effective population sizes [61].

The local evolution of TSR (Figure 3) also suggests that field population sizes are often so large that the probability of a *de novo* mutation is higher than the probability that a mutant allele is introduced via gene flow from another geographical region where resistant populations have evolved. NTSR likely also evolves locally from the standing variation available in each population [58]. However, once resistance has reached a high frequency in a given population, gene flow can propagate it to neighboring populations: the role of pollen flow in spreading resistance has been demonstrated in allogamous grass weeds [62,63], and wind dispersal of seeds of highly selfing *Conyza canadensis* was proposed to have contributed to rapid local spread of glyphosate resistance in the southern Central Valley of California [58].

Costs and benefits of resistance

Resistance mechanisms obviously confer a fitness advantage in the presence of a herbicide. Similarly to plant defense mechanisms, resistance mechanisms involve altered vital proteins (TSR) or secondary metabolism pathways (NTSR). It is widely accepted that such mechanisms have associated pleiotropic effects [64] that confer a fitness cost in the absence of the selective pressure [65]. One type of antagonistic pleiotropic effect is an increase in sensitivity to other herbicides (negative cross-resistance), as observed for TSR to herbicides in group K (e.g., [14]). A more striking example is an unexpected increase in sensitivity to herbicides in groups E and F associated with an allele conferring TSR to herbicides in group B [66]. With the exception of these few cases, negative cross-resistance has rarely been observed in weeds.

Pleiotropic effects of herbicide resistance mechanisms generally affect plant physiological and developmental processes [67]. Reliably assessing such pleiotropic effects is not straightforward because they vary with the genetic background and environmental conditions, for example, the deleterious effects of an acetyl-CoA carboxylase allele conferring resistance to group A herbicides on plant vegetative and reproductive output differed significantly among populations and were more pronounced under limiting environmental conditions [68]. A recent review [67] concluded that 75% of published studies investigating herbicide resistance pleiotropic effects are flawed because of the lack of control for genetic background. Accordingly, guidelines for studies of pleiotropic effects have been published [67,69].

Early fitness studies considered TSR to herbicides in group C and easily identified a strong deleterious pleiotropic effect on plant seed and biomass production, and on competitive ability, owing to a deficiency in photosynthesis associated with resistance (reviewed in [67]). Subsequent studies investigating other herbicide groups yielded mixed results (Table 2). Pleiotropic effects clearly vary with resistance gene and, sometimes, with TSR allele (Table 2). For a given gene or allele, pleiotropic effects can also vary with species. For instance, pleiotropic effects associated with the acetohydroxyacid synthase allele Trp-574-Leu, which confers TSR to group B herbicides, varies from a major reduction in biomass and competitive ability to no visible effect (Table 2).

Deleterious pleiotropic effects of resistance are not necessarily strong, which explains why resistance can persist in weed populations for years after the removal of any herbicide selective pressure (e.g., [70]). Moreover, pleiotropic effects of herbicide resistance can be beneficial, even in the absence of a herbicide. This is the case with acetyl-CoA carboxylase allele Ile-1781-Leu which confers TSR to herbicides in group A and causes an increase in weed biomass production [71] as well as a delay in seed germination that potentially allows escape from early-season weed control measures [72] (Table 2). Pleiotropic effects of this allele

Table 2. Pleiotropic effects associated with resistance to herbicides

Resistance mechanism[a]	Herbicide group	Species	Effect associated with herbicide resistance				Refs
			Seed germination	Vegetative biomass	Seed production	Competitive ability	
TSR, Ile-1781-Leu	A	*Alopecurus myosuroides*	Delay	None detected	None detected	Na[b]	[68,72]
TSR, Ile-2041-Asn	A	*Alopecurus myosuroides*	None detected	None detected	None detected	Na[b]	[68,72]
TSR, Gly-2078-Ala	A	*Alopecurus myosuroides*	Acceleration	Reduction	Reduction	Na[b]	[68,72]
TSR, Ile-1781-Leu	A	*Lolium sp.*	Delay	None detected	None detected	None detected	[73,88]
TSR, Ile-1781-Leu	A	*Setaria sp.*	Na[b]	Increase	Increase	Increase	[71]
TSR, Pro-197-Ala	B	*Lolium sp.*	Na[b]	None detected[c]	Na[b]	Na[b]	[89]
TSR, Pro-197-Arg	B	*Lolium sp.*	Na[b]	None detected[c]	Na[b]	Na[b]	[89]
TSR, Pro-197-Gln	B	*Lolium sp.*	Na[b]	None detected[c]	Na[b]	Na[b]	[89]
TSR, Pro-197-Ser	B	*Lolium sp.*	Na[b]	None detected[c]	Na[b]	Na[b]	[89]
TSR, Trp-574-Leu	B	*Lolium sp.*	Na[b]	None detected[c]	Na[b]	Na[b]	[89]
TSR, Pro-197-His	B	*Lactuca serriola*	Na[b]	Reduction	Reduction	Na[b]	[90]
TSR, Trp-574-Leu	B	*Amaranthus powellii*	Na[b]	Reduction	Reduction	Na[b]	[91]
NTSR	A, B	*Lolium sp.*	None detected	Reduction	Reduction	Reduction	[73,88,92]
NTSR	O	*Sinapis arvensis*	Na[b]	Reduction[c]	Reduction[c]	Reduction[c]	[93]
NTSR	G	*Lolium sp.*	Na[b]	Reduction[c]	Reduction[c]	Na[b]	[94]

[a]TSR allele indicated as wild type codon-codon position-mutant codon.

[b]Not assessed.

[c]Potential confounding effects due to genetic background cannot be excluded given the experimental procedure used.

thus enhance the selective advantage conferred by resistance, and this likely explains why this allele is by far the most widespread in weed populations that have evolved resistance to group A herbicides [28] (Figure 3).

The pleiotropic effects of many of the alleles that confer TSR and the pleiotropic effects of most of the NTSR mechanisms remain to be investigated, as are the pleiotropic effects of combinations of resistance genes and mechanisms. Individuals within weed populations can accumulate multiple resistance genes [34,35]. To date, however, a single study has examined combined pleiotropic effects [73]. Results of this study suggested partial compensation for the deleterious effects of a NTSR mechanism on weed growth rate by the beneficial effects of a TSR allele.

Most published studies of pleiotropic effects only investigated part of the life cycles of weed species (e.g., vegetative growth and seed output, or seed germination) (Table 2). However, a comprehensive understanding of the pleiotropic effects of herbicide resistance requires investigation of the entire weed life cycle, as has only been conducted to date for three TSR alleles [68,72].

Concluding remarks

In this review we have focused on the genetic bases of resistance and its evolution in response to selection by herbicides. Non-genetic factors that can also play a role in herbicide resistance evolution have been scarcely investigated to date. Clearly, environmental conditions influence plant physiology, and variation in the expression of herbicide resistance with temperature has recently been demonstrated [74]. Under global climate change, considering genetic × environment interactions could thus become increasingly important to predict and monitor accurately the evolution of herbicide resistance. Epigenetic processes are involved in the regulation of stress responses [75]. Transmission of pre-regulated expression patterns to the progeny of plants having survived herbicide stress without the need for mutations could accelerate the evolution of resistance. This topic opens entirely new research avenues for studies of herbicide resistance.

The diversity of herbicide resistance mechanisms observed today is the result of very rapid, but brief, evolution in response to herbicide selection pressure. Weed populations are undoubtedly still continuing to evolve such that the long-term outcome of herbicide selection is likely to differ from the situation today. Experimental evolution studies of the alga *Chlamydomonas reinhardtii* suggested that resistance may be less costly and more effective when it evolves by subtle genetic changes to many pathways than by large alterations to few pathways [76]. Thus, in the future, non-costly and broad-spectrum polygenic resistance to herbicides may predominate in weeds of agricultural fields.

Our literature survey suggests that the common distinction between TSR and NTSR has in itself no operational value for predicting the future of resistance evolution. Instead, the focus should be on whether resistance has a monogenic or polygenic basis, and whether it confers a 'specialist' or 'generalist' phenotype in terms of resistance to a single or multiple modes of herbicide action, respectively (*sensu* Vogwill [76]).

Among the hundreds of species of weeds that occur within cultivated fields, only a handful have repeatedly evolved resistance to multiple herbicides. These species are the grasses *Alopecurus myosuroides*, *Echinochloa crus-galli*, *Lolium multiflorum*, *L. rigidum*, and *Sorghum halepense*, and several species of the broadleaved weed genera *Amaranthus* and *Conyza*. Predicting which species will next become agriculturally and economically important herbicide-resistant weeds is crucial. Theoretical expectations from evolutionary rescue models highlight the importance of population size and standing genetic variation for evolution following rapid and major environmental change. The local abundance of a weed seems a good predictor of the probability it will evolve resistance. Among the 10 most-abundant weed species in winter wheat in France in the early 1970s [77], seven have evolved resistance to the herbicides subsequently used to control them. Obviously, more data are needed regarding standing genetic variation for resistance.

Proactive, evolutionary-based weed management options that integrate both herbicides and non-chemical tools are of utmost importance in agriculture today. Weed science should take advantage of the increasing focus and recent progress in understanding contemporary evolution processes in the field of evolutionary ecology [78]. This would undoubtedly boost integrative modeling efforts. Most current models are agronomy-, demography- or genetics-oriented, although new, promising modeling tools are emerging (e.g., [54]). A 'meta-model' that enables the unraveling and evaluation of the effects of the numerous, diverse factors involved, but is also capable of integrating all aspects of resistance evolution, is essential for understanding the adaptive process, designing resistance management strategies, and ultimately ensuring the long-term security of our food supply. This model remains to be built.

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References

1 Oerke, E-C. (2006) Crop losses to pests. *J. Agric. Sci.* 144, 31–43
2 Pimentel, D. *et al.* (2000) Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50, 53–65
3 Foster, D.K.P. *et al.* (1993) Selective grass-weed control in wheat and barley based on the safener fenchlorazole-ethyl. In *Proceedings of the Brighton Crop Protection Conference – Weeds* (British Crop Protection Council ed), pp. 1267–1272 British Crop Protection Council
4 Baastians, L. *et al.* (2008) Focus on ecological weed management: what is hindering adoption? *Weed Res.* 48, 481–491
5 Hilton, H.W. (1957) Herbicide tolerant strains of weeds. In *Hawaiian Sugar Planters Association Annual Report* (Hawaiian Sugar Planters Association ed), pp. 69–72, Hawaiian Sugar Planters Association
6 Duke, S.O. (2012) Why have no new herbicide modes of action appeared in recent years? *Pest Manag. Sci.* 68, 505–512
7 Jasieniuk, M. *et al.* (1996) The evolution and genetics of herbicide resistance in weeds. *Weed Sci.* 44, 176–193
8 Neve, P. *et al.* (2009) Evolutionary-thinking in agricultural weed management. *New Phytol.* 184, 783–793
9 Powles, S.B. and Yu, Q. (2010) Evolution in action: plants resistant to herbicides. *Annu. Rev. Plant Biol.* 61, 317–347
10 Beckie, H. and Tardif, F.J. (2012) Herbicide cross resistance in weeds. *Crop Prot.* 35, 15–28

11 Délye, C. (2013) Unravelling the genetic bases of non-target-site based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. *Pest Manag. Sci.* 69, 176–187
12 Avila-Garcia, W.V. *et al.* (2012) Target-site mutation associated with glufosinate resistance in Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*). *Pest Manag. Sci.* 68, 1248–1254
13 Shaner, D.L. *et al.* (2012) What have the mechanisms of resistance to glyphosate taught us? *Pest Manag. Sci.* 68, 3–9
14 Délye, C. *et al.* (2004) Molecular bases for sensitivity to tubulin-binding herbicides in green foxtail. *Plant Physiol.* 136, 3920–3932
15 Wang, J-G. *et al.* (2009) Crystal structures of two novel sulfonylurea herbicides in complex with *Arabidopsis thaliana* acetohydroxyacid synthase. *FEBS J.* 276, 1282–1290
16 Dayan, F.E. *et al.* (2010) Biochemical and structural consequences of a glycine deletion in the α-8 helix of protoporphyrinogen oxidase. *Biochim. Biophys. Acta* 1804, 1548–1556
17 Yu, L.P.C. *et al.* (2010) Mechanism for the inhibition of the carboxyltransferase domain of acetyl-coenzyme A carboxylase by pinoxaden. *Proc. Natl. Acad. Sci. U.S.A.* 107, 22072–22077
18 Délye, C. *et al.* (2005) Molecular bases for sensitivity to acetyl-coenzyme A carboxylase inhibitors in black-grass. *Plant Physiol.* 137, 794–806
19 Yu, Q. *et al.* (2007) Diversity of acetyl-coenzyme A carboxylase mutations in resistant *Lolium* populations: evaluation using clethodim. *Plant Physiol.* 145, 547–558
20 Leroux, P. and Walker, A.S. (2011) Multiple mechanisms account for resistance to sterol 14α-demethylation inhibitors in field isolates of *Mycosphaerella graminicola*. *Pest Manag. Sci.* 67, 44–59
21 Karasov, T. *et al.* (2010) Evidence that adaptation in *Drosophila* is not limited by mutation at single sites. *PLoS Genet.* 6, e1000924
22 Patzoldt, W.L. *et al.* (2006) A codon deletion confers resistance to herbicides inhibiting protoporphyrinogen oxidase. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12329–12334
23 Han, H. *et al.* (2012) A novel amino acid substitution Ala-122-Tyr in ALS confers high-level and broad resistance across ALS-inhibiting herbicides. *Pest Manag. Sci.* 68, 1164–1170
24 Jalaludin, A. *et al.* (2013) Evolution in action: a double amino acid substitution in the EPSPS gene endows high-level glyphosate resistance. In *Global herbicide Resistance Challenge* (Powles, S. and Mayer, L., eds), p. 39, Australian Herbicide Resistance Initiative
25 Gaines, T.A. *et al.* (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1029–1034
26 Salas, R.A. *et al.* (2012) EPSPS gene amplification in glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) from Arkansas. *Pest Manag. Sci.* 68, 1223–1230
27 Pritchard, J.K. *et al.* (2010) The genetics of human adaptation: hard sweeps, soft sweeps, and polygenic adaptation. *Curr. Biol.* 20, R208–R215
28 Délye, C. *et al.* (2010) Geographical variation in resistance to acetyl-coenzyme A carboxylase-inhibiting herbicides across the range of the arable weed *Alopecurus myosuroides* (black-grass). *New Phytol.* 186, 1005–1017
29 Délye, C. *et al.* (2011) Evolution and diversity of the mechanisms endowing resistance to herbicides inhibiting acetolactate-synthase (ALS) in corn poppy (*Papaver rhoeas* L.). *Plant Sci.* 180, 333–342
30 Mithila, J. *et al.* (2011) Evolution of resistance to auxinic herbicides: historical perspectives, mechanisms of resistance, and implications for broadleaf weed management in agronomic crops. *Weed Sci.* 59, 445–457
31 Petit, C. *et al.* (2010) Prevalence of cross- or multiple resistance to the acetyl-coenzyme A carboxylase inhibitors fenoxaprop, clodinafop and pinoxaden in black-grass (*Alopecurus myosuroides* Huds.) in France. *Pest Manag. Sci.* 66, 168–177
32 Yuan, J.S. *et al.* (2007) Non-target-site herbicide resistance: a family business. *Trends Plant Sci.* 12, 6–13
33 Cummins, I. *et al.* (2009) Safener responsiveness and multiple herbicide resistance in the weed black-grass (*Alopecurus myosuroides*). *Plant Biotechnol. J.* 7, 807–820
34 Petit, C. *et al.* (2010) Complex genetic control of non-target-site-based resistance to herbicides inhibiting acetyl-coenzyme A carboxylase and acetolactate-synthase in *Alopecurus myosuroides* Huds. *Plant Sci.* 178, 501–509
35 Busi, R. *et al.* (2011) Genetic control of a cytochrome P450 metabolism-based herbicide resistance mechanism in *Lolium rigidum*. *Heredity* 106, 817–824
36 Mithila, J. *et al.* (2012) Development of near-isogenic lines and identification of markers linked to auxinic herbicide resistance in wild mustard (*Sinapis arvensis* L.). *Pest Manag. Sci.* 68, 548–556
37 Cummins, I. *et al.* (1999) A role for glutathione transferases functioning as glutathione peroxidases in resistance to multiple herbicides in black-grass. *Plant J.* 18, 285–292
38 Cummins, I. *et al.* (2013) Key role for a glutathione transferase in multiple-herbicide resistance in grass weeds. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5812–5817
39 Cummins, I. and Edwards, R. (2004) Purification and cloning of an esterase from the weed black-grass (*Alopecurus myosuroides*), which bioactivates aryloxyphenoxypropionate herbicides. *Plant J.* 39, 894–904
40 Peng, Y. *et al.* (2010) Characterization of the horseweed (*Conyza canadensis*) transcriptome using GS-FLX 454 pyrosequencing and its application for expression analysis of candidate non-target herbicide resistance genes. *Pest Manag. Sci.* 66, 1053–1062
41 Riggins, C.W. *et al.* (2010) Characterization of de novo transcriptome for waterhemp (*Amaranthus tuberculatus*) using GS-FLX 454 pyrosequencing and its application for studies of herbicide target-site genes. *Pest Manag. Sci.* 66, 1042–1052
42 Lai, Z. *et al.* (2012) Genomics of Compositae weeds: EST libraries, microarrays, and evidence of introgression. *Am. J. Bot.* 99, 209–218
43 Zhang, Y. *et al.* (2012) De novo foliar transcriptome of *Chenopodium amaranticolor* and analysis of its gene expression during virus-induced hypersensitive response. *PLoS ONE* 7, e45953
44 Neve, P. and Powles, S. (2005) Recurrent selection with reduced herbicide rates results in the rapid evolution of herbicide resistance in *Lolium rigidum*. *Theor. Appl. Genet.* 110, 1154–1166
45 Norsworthy, J.K. *et al.* (2008) Response of northeastern Arkansas Palmer amaranth (*Amaranthus palmeri*) accessions to glyphosate. *Weed Technol.* 22, 408–413
46 Busi, R. and Powles, S. (2009) Evolution of glyphosate resistance in a *Lolium rigidum* population by glyphosate selection at sublethal doses. *Heredity* 103, 318–325
47 Busi, R. and Powles, S. (2011) Reduced sensitivity to paraquat evolves under selection with low glyphosate doses in *Lolium rigidum*. *Agron. Sustain. Dev.* 31, 525–531
48 Manalil, S. *et al.* (2011) Rapid evolution of herbicide resistance by low herbicide dosages. *Weed Sci.* 59, 210–217
49 Busi, R. *et al.* (2012) Understanding the potential for resistance evolution to the new herbicide pyroxasulfone: field selection at high doses versus recurrent selection at low doses. *Weed Res.* 52, 489–499
50 Brotherton, J.E. *et al.* (2007) Identification of *Arabidopsis thaliana* variants with differential glyphosate responses. *J. Plant Physiol.* 164, 1337–1345
51 Neve, P. and Powles, S. (2005) High survival frequencies at low herbicide use rates in populations of *Lolium rigidum* result in rapid evolution of herbicide resistance. *Heredity* 95, 485–492
52 Smith, D.A. and Hallett, S.G. (2006) Variable response of common waterhemp (*Amaranthus rudis*) populations and individuals to glyphosate. *Weed Technol.* 20, 466–471
53 Gonzalez, A. *et al.* (2013) Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. *Philos. Trans. R. Soc. B* 368, 20120404
54 Renton, M. *et al.* (2011) Does cutting herbicide rates threaten the sustainability of weed management in cropping systems? *J. Theor. Biol.* 283, 14–27
55 Loureiro, I. *et al.* (2010) Distribution and frequency of resistance to four herbicide modes of action in *Lolium rigidum* Gaud. accessions randomly collected in winter cereal fields in Spain. *Crop Prot.* 29, 1248–1256
56 Espeby, L.A. *et al.* (2011) Susceptibility variation to new and established herbicides: Examples of inter-population sensitivity of grass weeds. *Crop Prot.* 30, 429–435
57 Escorial, C. *et al.* (2011) Population variability in the response of ripgut brome (*Bromus diandrus*) to sulfosulfuron and glyphosate herbicides. *Weed Sci.* 59, 107–112
58 Okada, M. *et al.* (2013) Evolution and spread of glyphosate resistance in *Conyza canadensis* in California. *Evol. Appl.* http://dx.doi.org/10.1111/eva.12061

59 Délye, C. *et al.* (2004) Nucleotide variability at the acetyl-coenzyme A carboxylase gene and the signature of herbicide selection in the grass weed *Alopecurus myosuroides* Huds. *Mol. Biol. Evol.* 21, 884–892
60 Barton, N. (2010) Understanding adaptation in large populations. *PLoS Genet.* 6, e1000987
61 Lundemo, S. *et al.* (2009) Seed banks cause elevated generation times and effective population sizes of *Arabidopsis thaliana* in northern Europe. *Mol. Ecol.* 18, 2798–2811
62 Délye, C. *et al.* (2010) High gene flow promotes the genetic homogeneity of arable weed populations at the landscape level. *Basic Appl. Ecol.* 11, 504–512
63 Busi, R. *et al.* (2011) Gene flow increases the initial frequency of herbicide resistance alleles in unselected *Lolium rigidum* populations. *Agric. Ecosyst. Environ.* 142, 403–409
64 Pavlicev, M. and Wagner, G.P. (2012) A model of developmental evolution: selection, pleiotropy and compensation. *Trends Ecol. Evol.* 27, 316–322
65 Purrington, C.B. (2000) Costs of resistance. *Curr. Opin. Plant Biol.* 3, 305–308
66 Beckie, H.J. *et al.* (2012) Negative cross-resistance of acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) to protoporphyrinogen oxidase- and hydroxyphenylpyruvate dioxygenase-inhibiting herbicides. *Weed Technol.* 26, 570–574
67 Vila-Aiub, M.M. *et al.* (2009) Fitness costs associated with evolved herbicide resistance alleles in plants. *New Phytol.* 184, 751–767
68 Menchari, Y. *et al.* (2008) Fitness costs associated with three mutant acetyl-coenzyme A carboxylase alleles endowing herbicide resistance in black-grass *Alopecurus myosuroides*. *J. Appl. Ecol.* 45, 939–947
69 Vila-Aiub, M.M. *et al.* (2011) A unified approach to the estimation and interpretation of resistance costs in plants. *Heredity* 107, 386–394
70 Chauvel, B. *et al.* (2009) Evolution of a herbicide-resistant population of *Alopecurus myosuroides* Huds. in a long-term cropping system experiment. *Crop Prot.* 28, 343–349
71 Wang, T. *et al.* (2010) A herbicide-resistant ACCase 1781 *Setaria* mutant shows higher fitness than wild type. *Heredity* 105, 394–400
72 Délye, C. *et al.* (2013) A new insight into arable weed adaptive evolution: mutations endowing herbicide resistance also affect germination dynamics and seedling emergence. *Ann. Bot.* 111, 681–691
73 Vila-Aiub, M.M. *et al.* (2005) Resistance cost of a cytochrome P450 herbicide metabolism mechanism but not an ACCase target site mutation in a multiple resistant *Lolium rigidum* population. *New Phytol.* 167, 787–796
74 Vila-Aiub, M.M. *et al.* (2013) Glyphosate resistance in *Sorghum halepense* and *Lolium rigidum* is reduced at suboptimal growing temperatures. *Pest Manag. Sci.* 69, 228–232
75 Boyko, A. and Kovalchuk, I. (2008) Epigenetic control of plant stress response. *Environ. Mol. Mutagen.* 49, 61–72
76 Vogwill, T. *et al.* (2012) The experimental evolution of herbicide resistance in *Chlamydomonas reinhardtii* results in a positive correlation between fitness in the presence and absence of herbicides. *J. Evol. Biol.* 25, 1955–1964
77 Fried, G. *et al.* (2012) Trajectories of weed communities explained by traits associated with species' response to management practices. *Agric. Ecosyst. Environ.* 158, 147–155
78 Hendry, A.P. *et al.* (2011) Evolutionary principles and their practical application. *Evol. Appl.* 4, 159–183
79 Beckie, H.J. and Reboud, X. (2009) Selecting for weed resistance: herbicide rotation and mixture. *Weed Technol.* 23, 363–370
80 Neve, P. *et al.* (2011) Modelling evolution and management of glyphosate resistance in *Amaranthus palmeri*. *Weed Res.* 51, 99–112
81 Lagator, M. *et al.* (2013) Herbicide mixtures at high doses slow the evolution of resistance in experimentally evolving populations of *Chlamydomonas reinhardtii*. *New Phytol.* 198, 938–945
82 Beckie, H.J. (2006) Herbicide-resistant weeds: management tactics and practices. *Weed Technol.* 20, 793–814
83 Jacquemin, B. *et al.* (2008) Modelling binary mixtures of herbicides in populations resistant to one of the components: evaluation for resistance management. *Pest Manag. Sci.* 65, 113–121
84 Green, J.M. (2012) The benefits of herbicide-resistant crops. *Pest Manag. Sci.* 68, 1323–1331
85 Harker, K.N. *et al.* (2012) Our view. *Weed Sci.* 60, 143–144
86 Orr, H.A. and Unckless, R.L. (2008) Population extinction and the genetics of adaptation. *Am. Nat.* 172, 160–169
87 Gomulkiewicz, R. *et al.* (2010) Genetics, adaptation, and invasion in harsh environments. *Evol. Appl.* 3, 97–108
88 Vila-Aiub, M.M. *et al.* (2005) Ecological fitness of a multiple herbicide-resistant *Lolium rigidum* population: dynamics of seed germination and seedling emergence of resistant and susceptible phenotypes. *J. Appl. Ecol.* 42, 288–298
89 Yu, Q. *et al.* (2010) AHAS herbicide resistance endowing mutations: effect on AHAS functionality and plant growth. *J. Exp. Bot.* 61, 3925–3934
90 Alcocer-Ruthling, M. *et al.* (1992) Differential competitiveness of sulfonylurea resistant and susceptible prickly lettuce (*Lactuca serriola*). *Weed Technol.* 6, 303–309
91 Tardif, F.J. *et al.* (2006) A mutation in the herbicide target site acetohydroxyacid synthase produces morphological and structural alterations and reduces fitness in *Amaranthus powellii*. *New Phytol.* 169, 251–264
92 Vila-Aiub, M.M. *et al.* (2009) Evidence for an ecological cost of enhanced herbicide metabolism in *Lolium rigidum*. *J. Ecol.* 97, 772–780
93 Hall, J.C. and Romano, M.L. (1995) Morphological and physiological differences between the auxinic herbicide-susceptible (S) and herbicide-resistant (R) wild mustard (*Sinapis arvensis* L.) biotypes. *Pestic. Biochem. Physiol.* 52, 149–155
94 Pedersen, B.P. *et al.* (2007) Ecological fitness of a glyphosate-resistant *Lolium rigidum* population: growth and seed production along a competition gradient. *Basic Appl. Ecol.* 8, 258–268