

# Herbicide Resistant Weeds and Crops

Bradley D. Hanson,<sup>1</sup> Albert J. Fischer,<sup>2</sup> Alan McHughen,<sup>3</sup> Marie Jasieniuk,<sup>4</sup> Anil Shrestha,<sup>5</sup> and Amit J. Jhala<sup>6</sup>

1. University of California Cooperative Extension, Davis, CA

2. University of California, Davis

3. University of California Cooperative Extension, Riverside, CA

4. University of California, Davis

5. California State University, Fresno

6. University of Nebraska, Lincoln

Modern herbicidal weed control often exploits physiological differences among plants and takes advantage of herbicide tolerance or resistance in crop plants to bring about the selective removal of susceptible weeds. The Weed Science Society of America defines herbicide tolerance as “*the inherent ability of a plant to survive and reproduce after herbicide treatment*” which suggests that herbicide-tolerant plants have a natural level of tolerance not due to selection or genetic manipulation. In contrast, herbicide resistance is defined as “*the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type*”. This definition includes both naturally occurring cases of evolved resistance and those resulting from genetic engineering, mutagenesis or in-vitro techniques.

Differential tolerance to herbicides has been the fundamental basis for selective control of weeds in many crops. The 1940's discovery that auxinic herbicides primarily affect broadleaf plants while sparing grass crops (e.g., turf, cereals) ushered in the modern herbicide era. For decades, chemicals were tested for weed control and crop safety and hundreds of products were developed. In some cases, slight manipulations of functional groups on a base molecule resulted in changes in crop selectivity, weed control efficacy, and environmental persistence and led to whole new “families” of herbicides (e.g., triazines, sulfonyleureas, imidazolinones, thiocarbamates). This chemical screening approach has also been used to develop commercial uses for non-selective herbicides using differences in application timing or placement to selectively expose only weeds to the herbicide. More detail on the chemistry, activity, and development of specific herbicides, herbicide families, and mode of action groups can be found in other chapters of this book.

Selection pressures on weed populations have increased as weed control practices have become more effective and specific. In response to highly effective management practices, weed populations have shifted to more tolerant species or have evolved herbicide-resistant biotypes in several cropping systems.

Although naturally occurring herbicide tolerance in crops is the foundation on which modern weed control was built, technological advances in plant breeding and genetic engineering have allowed the development of new herbicide-resistant crop plants. Using techniques unavailable only a few decades ago, herbicide resistance traits from sexually incompatible species have been introduced into several crop plants. Herbicide-resistant crops have unarguably had a profound impact on chemical weed control practices and agricultural economics; however this technology is not without controversy.

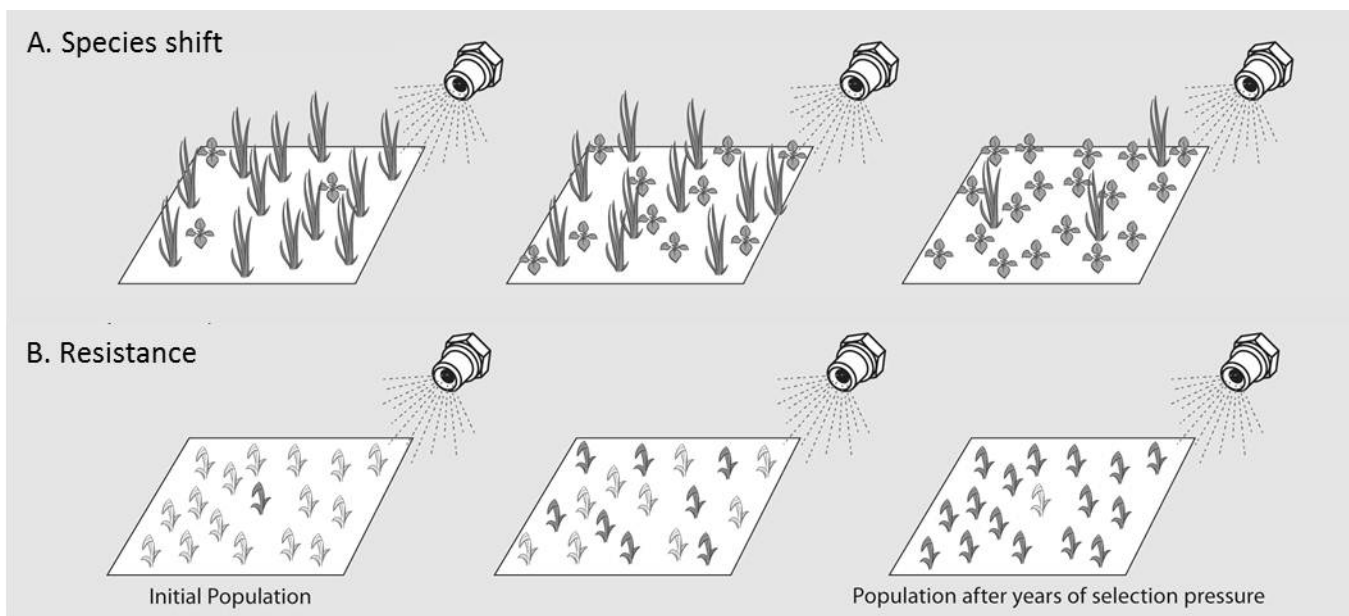
The issues and technology surrounding herbicide tolerance and resistance in weeds and crops are constantly changing and present farmers and land managers with many new opportunities as well as difficult weed management problems. This chapter attempts to introduce, discuss, and explain some of the impacts of herbicide tolerance and resistance from both a weed and crop standpoint.

## HERBICIDE TOLERANT AND RESISTANT WEEDS

Herbicides are used for selective and non-selective control of weeds in agricultural, commercial, industrial, and residential areas. Although herbicides can provide impressive levels of weed control in many situations, not all weedy species are equally controlled due to varying levels of natural tolerance or the evolution of herbicide-resistant weed biotypes.

Weedy plants can be tolerant of herbicides due to a variety of temporal, spatial, or physiological mechanisms. A weed that emerges after a burn down herbicide is applied, or completes its lifecycle before a post emergence herbicide is applied, avoids the control effort. Similarly, large-seeded or perennial weeds can emerge from deeper in the soil and, in some cases may grow through soil treated with a pre-emergence herbicide with minimal injury. Other weedy species have physiological mechanisms of tolerance and avoid control through reduced herbicide uptake, rapid detoxification, or insensitive target sites.

Regardless of the mechanism of tolerance, repeated use of an herbicide can lead to “weed shifts” in which weed populations change to a higher proportion of species that are not controlled by the weed management tactic used (Figure 1a). A classic example of a weed shift in response to herbicides is the change from primarily broadleaf weeds to grass weeds in cereal production after the introduction of the broadleaf herbicide 2,4-D. Weed shifts can also follow overuse of non-chemical weed control techniques. For example, flame weed control is more effective on broadleaf weeds and can result in shifts to grass weeds in organic vineyards. Similarly, repeated mowing (in turf or orchards) can shift weed populations from species with erect growth habits to more prostrate or decumbent habits like knotweed, common purslane, puncturevine, or spotted spurge.



**Figure 1.** Herbicides impose selection pressure and can lead to weed species shifts resulting in populations dominated by more tolerant species (a). Occasionally, an individual weed has a mutation that confers resistance to a herbicide or group of herbicides, and this individual survives and reproduces despite being treated with herbicide (b). In both cases, after several generations and repeated selection with the same or similar herbicides, the tolerant species or resistant biotype can become dominant in the population. Figure modified from UC ANR Publication #8362 with permission.

Herbicide resistance in weeds results from the process of adaptive evolution in weed populations and is due in large part to selection with herbicides—usually repeated use of the same herbicide or products with the same mode of action. Scientific studies have not substantiated the hypothesis that herbicides induce resistance mutations. Instead,

herbicide resistance appears to be due to selection on naturally occurring mutations in plants within weed populations. Naturally occurring mutations may be lethal to the individual (e.g. cause a plant to not produce chlorophyll), may be beneficial (e.g. confer drought tolerance) or may be neutral (i.e., have no observable effect on plant fitness). Occasionally, however, one of these chance mutations affects the target site of an herbicide such that the herbicide can no longer bind to the target site and thus does not affect the new biotype. Similarly, mutations can affect other plant processes in a way that reduces the plant's exposure to the herbicide due to reduced uptake or translocation or through more rapid detoxification. Whatever the cause, under continued "selection pressure" with the herbicide, resistant plants are not controlled and their progeny can build up in the population (**Figure 1b**). Depending on the initial frequency and genetic basis of resistance, the frequency and rate of herbicide applications, and the reproductive ability of the weed, it may take few or many generations for the resistance problem to become apparent.

### Mechanisms of herbicide resistance

Understanding the biological mechanisms underlying herbicide resistance provides a rational basis to design weed management strategies to mitigate the evolution of resistance. We can currently distinguish two types of mechanisms conferring resistance to herbicides in weeds. There are mechanisms related to the specific site of action of the herbicide in the plant and others that involve processes not related to the mechanism by which herbicides kill plants; these are known as *target site* and *non-target site* mechanisms, respectively. A certain weed biotype may be resistant to more than one herbicide. Herbicide *cross-resistance* occurs when an individual plant is resistant to two different herbicides via the same mechanism of resistance. In this case, resistance is endowed by a single physiological process operating in common for all the herbicides involved. This often occurs with herbicides that share the same mechanism of action such as cross-resistance among several herbicide classes that inhibit the acetolactate synthase enzyme (ALS) or among all herbicidal inhibitors of the acetyl coenzyme A carboxylase (ACCase). *Multiple resistance* results from selection by the simultaneous or sequential use of different herbicides, such that resistance to each herbicide is endowed by a different mechanism. It is difficult to choose alternative herbicides to control weeds that have evolved cross-resistance or multiple resistance thus this represents a serious challenge for weed control.

### Target-site resistance

The target site of an herbicide is usually a specific protein (enzyme) that plays a key role in a certain plant metabolic process whose impairment has lethal consequences to the plant. Target site resistance occurs when the target enzyme becomes less sensitive or insensitive to the herbicide. The loss of sensitivity is usually associated with point mutation(s) in the gene coding for the protein and can result in amino-acid substitutions in specific regions of the protein. The amino acid substitutions can lead to conformational changes in the protein's structure which can impair the ability of one or more herbicides to attach to the specific binding site on the enzyme. Changes in protein structure occasionally result in reduced biological functionality of the enzyme and a related "fitness cost" of resistance; however, a measurable fitness cost has not been detected for many target site mutations [see Vila-Aiub et al. 2009 for a review]. Target site resistance has been well described for many herbicides. Certain herbicide groups are particularly vulnerable, because resistance can be endowed by several mutations [see Powles & Yu 2010 for a review], thus increasing the probability of finding resistant mutants in weed populations—even in those not previously exposed to that herbicide group. For example, specific mutations in the ALS gene resulting in seven different amino acid substitutions in the enzyme ALS are known to confer resistance to ALS-inhibiting herbicides in weed biotypes selected under field conditions. The ALS enzyme catalyzes the first committed step in the biosynthesis of branched-chain amino acids (valine, leucine and isoleucine). There are six chemical groups of herbicides that inhibit ALS: sulfonylureas (SU), imidazolinones (IMI), triazolopyrimidines (TP), pyrimidinylthiobenzoates (PTB), and sulfonylaminocarbonyltriazolinone (SCT). Depending on the specific mutation, a weed biotype resistant to SU often has cross-resistance to other SU herbicides. It may also happen that individuals with a mutation conferring SU-herbicide resistance are also cross-resistant to TP herbicides but susceptible to IMI. Thus several cross-resistance combinations may occur depending on the specific mutations and ALS amino acid substitutions (for a table of ALS gene mutations and cross-resistance patterns in different species see: Tranel et al. <http://www.weedscience.com>). Something similar occurs with the grass herbicides that inhibit the enzyme ACCase for which at least seven point mutations causing amino acid substitutions within the plastidic, homomeric

ACCase are associated with cross-resistance patterns observed at the whole plant level involving aryloxyphenoxy propionate (AOPP) and cyclohexanedione (CHD) herbicides and the phenylpyrazoline herbicide pinoxaden (for a table of mutations and cross-resistance see: <http://www.weedscience.org/summary/home.aspx>). The existence of so many mutations conferring resistance is one reason why resistance to these herbicides is frequently found and can evolve rapidly. Resistance to glyphosate can also be target-site mediated. This herbicide inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway. This is a key metabolic route where 20 to 30% of the carbon fixed by photosynthesis is circulated. Inhibition of EPSPS by glyphosate causes an increase in shikimate and leads to plant death by disrupting several key metabolic processes. Resistance to glyphosate results from point mutations in the EPSPS gene causing amino acid substitutions in the protein affecting the ability of glyphosate molecules to bind to the enzyme; the enzyme is thus less sensitive to inhibition by glyphosate. These mutations confer moderate levels of resistance, which are nevertheless enough to enable plants to survive and reproduce, and selection for resistance to occur when glyphosate is applied repeatedly. Nucleotide changes resulting in proline to serine, alanine, or threonine substitutions at EPSPS position 106 have been identified in glyphosate resistant mutants.

Target-site resistance can also involve elevated levels of enzyme activity expression in resistant plants; for example, natural herbicide resistance in certain legumes (*Lotus* spp.) or selected resistance by the repeated use of glyphosate in palmer amaranth or in horseweed can involve the presence of elevated levels of EPSPS. Overexpression of the enzyme targeted by the herbicide can result from posttranscriptional effects, expression regulation, or be due to gene duplication and/or amplification. In the case of the recently elucidated resistance to glyphosate in a palmer amaranth biotype from Georgia, the EPSPS enzyme activity was inhibited by glyphosate, but resistant plants had 5 to 160 times more copies of the gene than susceptible plants. The gene amplification was heritable and correlated with resistance to glyphosate.

### Non-target-site resistance

Several mechanisms confer resistance to herbicides without involving the active site of the herbicide in the plant, which often results in unpredictable resistance to many herbicides. Of these, probably best known is the case of metabolic resistance by which resistant biotypes have an enhanced ability to metabolically degrade the herbicide to less- or non-toxic forms compared to the wild susceptible individuals of the same species. Different processes can be involved, and to date, non-target-site herbicide resistance has been attributed to three groups of isozymes (cytochrome P450 monooxidases, glutathione transferases, and glycosyltransferases) and also recently associated with ABC (“ATP-binding cassette”) transporters, thus involving four large gene families (see Yuan et al. 2007). The cytochrome P450 monooxidases (P450) catalyze oxidative reactions typical of Phase I detoxification in which herbicide molecules acquire functional groups to react with Phase II enzymes. Phase II metabolism involves conjugation of thiols or sugars with a large hydrophilic molecule. Glutathione *S*-transferases and glycosyltransferases catalyze conjugation of the Phase I herbicide residue with the tripeptide glutathione (homogluthathione in the case of legumes) via the nucleophilic attack of an electronegative group or with a sugar moiety, respectively. Certain herbicides can conjugate directly without undergoing Phase I metabolism. These conjugates are recognized by Phase III transporters, such as the ABC transporters, and are actively transported across the tonoplast into the vacuole or to extracellular spaces where they are further degraded and immobilized (Phase IV). Research relating membrane ABC transporters with herbicide resistance is very recent; these proteins generally have one or two sites for binding ATP whose energy of hydrolysis is used in the active transport of molecules. Their function in plants is to compartmentalize a wide array of substrates from ions to macromolecules and xenobiotics. Phase I and II enzymes are ubiquitous in plants and are involved in diverse plant metabolic processes including those related to secondary metabolites. They thus fulfill a relevant role in plant defenses and tolerance to biotic and abiotic stresses. Non-target-site resistance may involve gene transcriptional regulation, enhanced enzyme activity, altered substrate specificity, or expression of specific membrane transporters. This type of resistance can involve multiple genes and confer resistance to a wide array of herbicides. A dramatic example of multifactorial non-target-site resistance in California is the case of late watergrass. A biotype of this species has evolved resistance to thiocarbamates (molinate and thioencarb), several ALS- and ACCase-inhibiting herbicides, clomazone, quinclorac and paraquat. Such complex resistances involve P450 and GST enzymes, tolerance to reactive forms of oxygen, insensitivity along



the auxin response pathway leading to ethylene biosynthesis, and an enhanced ability to detoxify endogenous cyanide. Most of these non-target-site resistance mechanisms are also present in cultivated plants and are the means by which many herbicides can be used selectively without injuring crops. Therefore, these types of resistant biotypes are not selected for by the repeated use of an herbicide or herbicides that kill weeds via a specific mechanism of action as discussed for target-site resistance. Non-target-site resistance can be selected for by intensive use of diverse and unrelated selective herbicides that are similarly effective on a certain weed species and share a detoxification pathway or a mechanism precluding their accumulation at the target site (exclusion or sequestration) that is relatively common in plants. Such complex multifactorial resistances suggest the enhanced expression of stress tolerance mechanisms in plants and pose very difficult weed management challenges, since they can involve several unrelated herbicides, even herbicides to which weed populations have not been exposed. Thus the management of non-target-site herbicide resistance often represents a greater challenge than the mitigation of target-site resistance for which choosing of alternative herbicides can be easier.

Reduced herbicide absorption and/or translocation can contribute to resistance in certain biotypes. In most cases, these have been accessory mechanisms that somewhat contribute towards resistance in addition to a major resistance mechanism. However, recent evidence suggests that changes in absorption and/or translocation are an important contributor to glyphosate resistance in several biotypes. The ambimobile (xylem and phloem-mobile) transport of glyphosate with accumulation in growing points is a major reason for its effectiveness, and it has been shown that alterations in glyphosate translocation within the plant can confer an even greater level of resistance than the currently known mutations at the EPSPS target site. Thus, in certain glyphosate-resistant biotypes of *Lolium* spp. (Australia) and horseweed (USA), glyphosate failed to accumulate in growing points and roots and tended to remain concentrated in the leaves rather than translocating throughout the plant. Similarly, resistance to paraquat in hare barley and in horseweed has been ascribed to greater herbicide sequestration in the vacuoles of resistant vs. susceptible plants. Alterations in the expression and function of membrane transporters have been suggested as mechanisms for these translocation or sequestration-type resistances, but the details are still to be fully elucidated.

### **Resistance evolution and spread**

In response to repeated herbicide treatment, many weed populations have changed in genetic composition, such that resistance alleles and resistant genotypes have increased in proportion over time, and the populations have become adapted to the intense selection imposed by herbicides. The following sections briefly describe the process of resistance evolution in weeds and the factors that determine its probability and rate. For the purposes of this discussion, a weed population is defined as all the individuals (seeds, seedlings, and adults) of a weed species that co-occur in space and time in a specific site (i.e. crop, domestic landscape, natural area) and are thus subject to the same weed and crop management practices.

### **Sources of genetic variation for resistance**

For resistance to increase in a weed population, genetic variation for resistance must be present when a herbicide is applied. At least a few plants must have, and express, a gene or genes that confer resistance to the herbicide by some mechanism. The major sources of resistance genes in weed populations are: (1) gene mutation and (2) gene dispersal via pollen and/or seed.

#### ***Gene mutation***

Gene mutation is a process by which a change occurs in the DNA sequence of a gene within reproductive tissues (pollen or the ovule). Gene mutations occur spontaneously, randomly, and continually throughout the genome of all biological organisms, including plants. Occasionally, mutations at specific gene loci may result in a new character or trait (e.g., resistance to a specific herbicide) in the next generation of seeds and seedlings that may provide genetic resistance to a specific herbicide. Initially, only a few plants in a population are likely to have the resistance mutation and express resistance when the herbicide is applied. However, those few resistant plants surviving herbicide treatment will produce seeds while treated susceptible plants will not (Figure 1). Thus, the resistance genes will be transmitted

to offspring and the proportion of resistant plants will continue to increase in the population as long as the herbicide is applied.

### *Pollen or seed dispersal*

Dispersal of pollen and/or seed that carry resistance genes from resistant plants in a separate population may also provide a source of resistance genes in a susceptible population. Resistance genes may disperse naturally by pollen via wind, insects, or other pollination vectors. Resistance genes may also be spread by seed. Seed dispersal among weed populations can occur by natural vectors, such as wind, water, insect herbivores, mammals, etc., as well as by humans. For example, farmers commonly spread herbicide-resistant seeds from field to field on seeding, tillage, and/or harvesting equipment or by planting crop seed contaminated with resistant weed seed. Not surprisingly, the spread of resistance genes by pollen is more common for weed species that are highly outcrossing, such as the annual and self-incompatible weedy ryegrasses (*Lolium* spp.), which normally produce a lot of pollen. In contrast, for highly selfing weed species, such as horseweed and hairy fleabane, resistance genes are primarily spread by seeds.

Understanding the relative contribution of independent gene mutation versus gene flow by pollen and/or seed dispersal in resistance spread is important because it determines the success of different management practices (e.g., reducing the intensity of herbicide selection versus limiting the movement of resistant seed) in preventing the spread of resistance to adjacent sites across the landscape.

### **Selection by herbicides**

Herbicides impose strong selection pressures on weeds. Most are applied at rates that kill 90 to 99% of the susceptible plants of a species under optimum conditions. If resistance genes are present in a weed population due to mutation or gene flow, even at very low frequencies, repeated herbicide treatment will select for resistant plants and rapidly increase their numbers until they become dominant in the field, orchard, or vineyard being treated (Figures 1 and 2). The more effective an herbicide is in killing susceptible plants, the more rapid will be the shift to a population dominated by resistant plants when the herbicide is applied. Several herbicide characteristics and use patterns result in a higher mortality of susceptible plants than others, and thus impose stronger selection for resistance (Table 1). Among others, these characteristics include a single target site, highly specific mechanism of action, long-term soil residual activity, and frequent application.



**Figure 2.** Glyphosate-resistant horseweed in a peach orchard near Dinuba, CA. Photo courtesy of A. Shrestha.

**TABLE 1.** FACTORS INCREASING THE PROBABILITY AND RATE OF HERBICIDE RESISTANCE EVOLUTION AND SPREAD IN WEEDS.**Cropping system characteristics**

- Little or no crop rotation
- Little or no preplant or in-season tillage
- Low crop competition
- Little or no rotation of herbicide modes of action (MOA)

**Weed characteristics**

- Annual growth habit
- High seed production
- Little seed dormancy
- Some seed longevity in seedbank
- High frequency of resistance alleles or mutations
- Multiple generations per year
- Mechanisms for gene flow (pollen or seed)
- No fitness penalty for resistance trait
- Species is highly susceptible to the herbicide

**Herbicide characteristics**

- Single site of action
- High efficacy
- High use rate relative to amount needed (favors selection of target-site resistance)
- Low or marginally effective use rates (favors selection of non-target-site resistance)
- Long soil residual activity
- High frequency of use

Mathematical models have been used to predict how alternative herbicide use patterns and weed management practices might reduce the intensity of herbicide selection and thereby reduce the probability and rate of resistance evolution and spread. The models have shown that herbicide rotations and mixtures, and integrated weed management practices, can significantly slow the evolution and spread of resistance. When herbicides are used in rotation with cultural practices, selection for resistance to a particular herbicide is generally restricted to the growing seasons during which the herbicide is applied. With the exception of those herbicides having long residual activity, the frequency of resistant plants is unlikely to increase more rapidly than the frequency of susceptible plants during the “herbicide-off” periods, and may even decline if resistant plants have reduced fitness in the absence of herbicide (see discussion below). Thus, the probability of resistance evolution will be reduced, and the time to reach a given level of resistance to a specific herbicide will be extended, by rotating the herbicide with various cultural practices. Similarly, tank mixes and rotations consisting of herbicides differing in mode of action have been predicted to delay the onset of target-site re-

sistance to the individual herbicides. However, a drawback of using tank mixes and rotations of herbicides differing in mode of action as strategies to delay resistance is that these strategies can select for multiple resistance and non-target site resistance. Rotating herbicides with cultural practices and implementing integrated weed management practices are currently believed to be more effective approaches for managing resistance.

### **Other factors influencing resistance evolution and spread**

In addition to selection by herbicide and the frequency of resistance genes in weed populations, several other factors affect the probability and rate of resistance evolution in weeds. These factors include the nature of inheritance of resistance, the fitness of resistant plants in the absence of the selective herbicide, and the mating system and life history of a weed species.

#### *Inheritance of resistance*

Resistance to most classes of herbicides is determined by nuclear inheritance whereby resistance genes are transmitted to offspring by both pollen and ovules. In the majority of weeds in which the inheritance of resistance has been studied, resistance is controlled by a single, major nuclear gene. Additionally, in most species, resistance is conferred by a dominant, or partially dominant, allele. Mathematical models have shown that resistance conferred by a single, dominant allele results in more rapid evolution of resistance in outcrossing weeds than resistance conferred by a single, recessive allele. When a resistance mutation first appears in a weed population, it is rare. If the mutation is recessive, only the homozygotes will be resistant. However, if the resistance mutation is dominant, both homozygotes and heterozygotes will be resistant and capable of surviving herbicide treatment. Thus, a rare dominant resistance allele is much more likely to become established in an outcrossing weed population following herbicide selection.

In contrast to resistance to most other herbicides, resistance to the triazine herbicides is usually inherited cytoplasmically in weeds. In this case, the gene conferring triazine resistance is located in the chloroplast genome and resistance alleles are only transmitted to offspring by the maternal parent. Because all seeds produced by a resistant maternal plant will be resistant, repeated application of triazine herbicides in a population with a newly arisen resistance mutation will quickly result in the selection of resistant plants and resistance alleles.

#### *Fitness in the absence of herbicide treatment*

Resistant plants have sometimes been found to have lower fitness (survival, growth, and/or seed production successes) than susceptible plants in the absence of herbicide treatment. The reduced fitness of resistant plants during periods when herbicides are not applied will slow resistance evolution and spread, and increase the effectiveness of rotating herbicides with cultural practices in managing resistance. If resistant and susceptible plants have equal fitness, the relative frequency of resistant plants neither increases nor decreases but remains constant during herbicide-off periods. In contrast, if resistant plants suffer a fitness reduction relative to susceptible plants in the absence of herbicide treatment, the reduced fitness causes a decrease in resistant individuals in the population during the herbicide-off periods.

#### *Mating system and life span*

The mating system and life span of a weed species also influence the rate of evolution and spread of resistance. In highly outcrossing species, e.g. those that must receive pollen from other plants to produce fertile seed, such as rigid and Italian ryegrass, dominant resistance mutations have a much higher likelihood of establishment and spread than recessive resistance mutations. In outcrossing weeds, a newly arisen resistance mutation or immigrant resistance gene will initially be present in heterozygotes because most individuals in the population are susceptible homozygotes for that gene. If the mutation is dominant, the heterozygous plants will survive herbicide treatment. However, if the mutation is recessive, heterozygotes will be susceptible and thus will not survive herbicide treatment. A recessive resistance mutation is thus unlikely to spread in response to herbicide selection in outcrossing species in comparison with a resistance gene with some degree of dominant expression. Selection strongly favors dominant beneficial mutations in predomi-

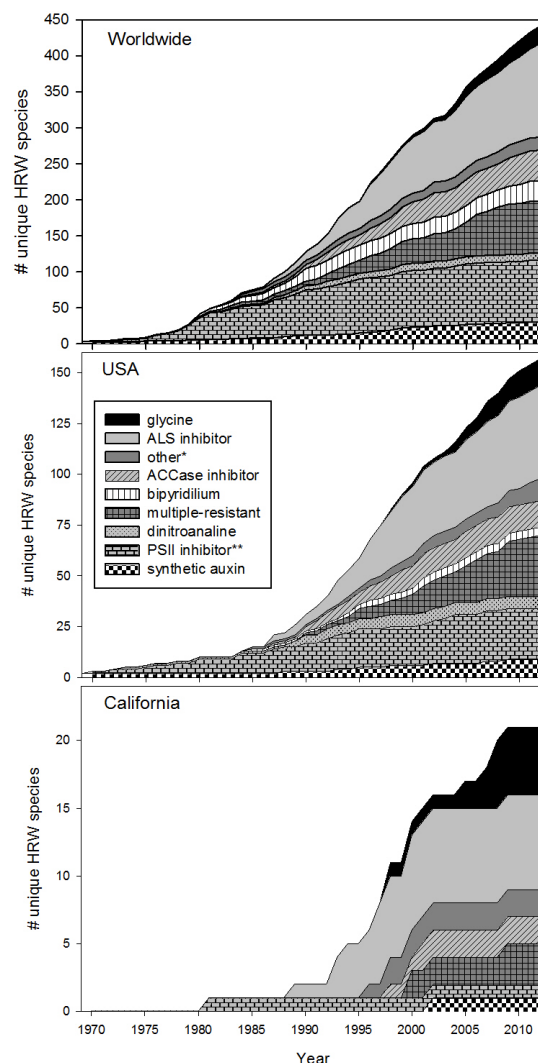


**Figure 3.** Chronological increase in reports of herbicide-resistant weeds in California, the USA, and worldwide. Data compiled from the International Survey of Resistant Weeds ([www.weedscience.org](http://www.weedscience.org)).

nantly outcrossing species. In contrast, in highly self-fertilizing weeds, e.g. those that pollenate their own ovules such as green foxtail, recessive resistance mutations can readily become established and spread. Selfing rapidly increases the frequency of homozygotes, at the expense of heterozygotes, thereby exposing recessive resistance alleles to herbicide selection and reducing the chance of loss of a newly arisen homozygous recessive mutation. In highly self-fertilizing species, therefore, recessive resistance mutations spread at roughly the same rate as dominant resistance mutations.

**Current status of herbicide resistance in weeds**

Herbicide-resistant weeds are an issue around the world; but the greatest problems with resistance tend to be found in countries with highly mechanized agricultural cropping systems due to the greater reliance on herbicides. According to the International Survey of Resistant Weeds (online at <http://www.weedscience.org>), herbicide-resistant weed biotypes have been reported in at least 60 countries and include about 400 unique species-herbicide group combinations (Figure 3). Due to intensive, high-input cropping systems, the United States has more resistant biotypes (144) than any other country followed by Australia (62), Canada (59), France, China, Germany, Spain, and Brazil (31–35). California has 21 unique cases of herbicide-resistant weeds including 15 grasses or sedges and 6 broadleaf species (Table 2).



**TABLE 2.** CONFIRMED CASES OF HERBICIDE-RESISTANT WEEDS IN CALIFORNIA

Scientific name	Common name	Year	Mode of action*
<i>Senecio vulgaris</i>	common groundsel	1981	PSII inhibitor
<i>Lolium perenne</i>	perennial ryegrass	1989	ALS inhibitor
<i>Cyperus difformis</i>	smallflower umbrella sedge	1993	ALS inhibitor
<i>Sagittaria montevidensis</i>	California arrowhead	1993	ALS inhibitor
<i>Salsola iberica</i>	Russian thistle	1994	ALS inhibitor
<i>Avena fatua</i>	wild oat	1996	pyrazolium (difenzoquat)
<i>Ammania auriculata</i>	redstem	1997	ALS inhibitor
<i>Scirpus mucronatus</i>	ricefield bulrush	1997	ALS inhibitor
<i>Echinochloa phyllopogon</i>	late watergrass	1998	multiple (ACCase inhibitor, ALS inhibitor, and thiocarbamate)
<i>Lolium rigidum</i>	rigid ryegrass	1998	glycine
<i>Ammania coccinea</i>	long-leaved loosestrife	2000	ALS inhibitor
<i>Echinochloa crus-galli</i>	barnyardgrass	2000	multiple (ACCase inhibitor and thiocarbamate)

Scientific name	Common name	Year	Mode of action*
<i>Echinochloa oryzicola</i>	early watergrass	2000	multiple (ACCase inhibitor and thiocarbamate)
<i>Phalaris minor</i>	small-seeded canarygrass	2001	ACCCase inhibitor
<i>Digitaria ischaemum</i>	smooth crabgrass	2002	synthetic auxin
<i>Conyza canadensis</i>	horseweed	2005	glycine
<i>Lolium multiflorum</i>	Italian ryegrass	2005	glycine
<i>Conyza bonariensis</i>	hairy fleabane	2007	glycine
<i>Echinochloa colona</i>	junglerice	2008	glycine
<i>Conyza bonariensis</i>	hairy fleabane	2009	multiple (glycine and bipyridilium)
<i>Cyperus difformis</i>	smallflower umbrella sedge	2013	PSII

\* PSII = photosystem II, ALS = acetolactate synthase, ACCCase = acetyl coenzyme A carboxylase.

Herbicide-resistant weeds around the world and throughout the U.S. are dominated by resistance to the photosystem II inhibitors and by ALS inhibitors due to the widespread use of these diverse herbicide classes in broad acreage cereal and grain crops. Some of the most troubling herbicide-resistant biotypes are multiple resistant biotypes—one population of rigid ryegrass in Australia is reported to be resistant to 9 different modes of action! In California, herbicide-resistant biotypes are dominated by ALS inhibitor resistance, primarily due to intense selection pressure in rice cropping systems. However, in recent years, glycine resistance (i.e., glyphosate resistance) and multiple resistant biotypes have become an increasing problem in the state.

Although herbicide resistance has been reported in many weedy species, a few genera have dominated documentation. Seventeen genera have been reported to have biotypes resistant to four or more herbicide mode of action groups (single- or multiple-resistance) (Table 3). These weeds tend to be among the most widespread problematic species and effective management is often further complicated by herbicide resistance.

**TABLE 3. WEED GENERA WITH HIGH PROPENSITY TO DEVELOP HERBICIDE RESISTANCE.**

Genus	Worldwide resistance reports		U.S. resistance reports	
	# species	MOA*	# species	MOA*
<i>Alopercurus</i>	2	ACC, ALS, chloroacet., DNA, PSII, urea	0	—
<i>Amaranthus</i>	8	ALS, bipyridilium, CBI, DNA, glycine, nitrile, PSII, PPO, urea	6	ALS, bipyridilium, CBI, DNA, glycine, PSII, PPO, urea
<i>Ambrosia</i>	2	ALS, glycine, PSII, PPO, urea	2	ALS, glycine, PSII, PPO
<i>Avena</i>	3	ACC, arylaminopropionic, ALS, DNA, other, thiocarbamate, unknown	1	ACC, ALS, DNA, thiocarbamate, unknown
<i>Chenopodium</i>	5	ALS, PSII, sythetic auxin, urea	2	ALS, PSII
<i>Conyza</i>	3	ALS, bipyridilium, glycine, PSII, urea	2	ALS, bipyridilium, glycine, PSII, urea
<i>Digitaria</i>	4	ACC, glycine, PSII, sythetic auxin	2	ACC, sythetic auxin

**TABLE 3.** WEED GENERA WITH HIGH PROPENSITY TO DEVELOP HERBICIDE RESISTANCE.  
(continued)

Genus	Worldwide resistance reports		U.S. resistance reports	
	# species	MOA*	# species	MOA*
<i>Echinochloa</i>	6	ACC, ALS, cellulose inhib., chloroacet., DNA, glycine, PSII, synthetic auxin, thiocarbamate, urea	3	ACC, PSII, synthetic auxin, thiocarbamate, urea, glycine
<i>Eleusine</i>	1	ACC, ALS, bipyridilium, DNA, glycine	1	bipyridilium, DNA
<i>Euphorbia</i>	1	ALS, glycine, PPO, urea	0	—
<i>Kochia</i>	1	ALS, glycine, PSII, synthetic auxin	1	ALS, glycine, PSII, synthetic auxin
<i>Lolium</i>	4	ACC, ALS, bipyridilium, chloroacet., DNA, glycine, mitosis inhib., nitrile, PSII, thiocarbamate, triazole, urea	4	ACC, ALS, chloroacet., glycine
<i>Phalaris</i>	3	ACC, ALS, PSII, urea	1	ACC
<i>Poa</i>	1	bipyridilium, DNA, PSII, thiocarbamate, triazole, urea	1	DNA, PSII, thiocarbamate, triazole, urea
<i>Raphanus</i>	2	ALS, CBI, PSII, synthetic auxin	0	—
<i>Setaria</i>	5	ACC, ALS, DNA, PSII	4	ACC, ALS, DNA, PSII
<i>Sorghum</i>	3	ACC, ALS, DNA, glycine	2	ACC, ALS, DNA, glycine

\* MOA=herbicide mode of action; ACCase = acetyl coenzyme A carboxylase, ALS = acetolactate synthase, CBI—cellulose biosynthesis inhibitor, DNA = dinitroaniline, chloroacet = chloracetamide, PPO = protoporphyrinogen IX oxidase, PSII = photosystem II.

## MANAGEMENT OF HERBICIDE-RESISTANT WEEDS

A number of factors affect the probability and rate of herbicide resistance evolution and spread in weeds (Table 1). However, if preventive measures are taken to reduce the selection pressure imposed by herbicides, resistance may be avoided or delayed. As outlined in previous sections, repeated use of the same herbicide or herbicides with the same mode of action selects for weeds that are resistant to that mode of action. As an herbicide controls the susceptible biotypes, with repeated use of the same herbicide, the resistant biotype gradually builds up in the population (Figure 1). The adage ‘why fix something that is not broken’ may not be applicable to herbicide resistance management. For example, it is tempting to repeatedly use a certain herbicide that is very effective against a certain weed species or community of weeds. However, such a process imposes a higher selection pressure on the target weed and can ultimately lead to a buildup of resistant plants. Therefore, a major goal of herbicide resistance management is to reduce selection pressure. In this context ‘rotation’ becomes a key word in herbicide resistance management and is often used as the first line of defense against the selection of herbicide-resistant weeds.

Rotation can be in terms of use of different crops in a sequence as in the case of annual cropping systems (e.g. wheat-cotton-wheat). In this particular cropping system, a broadleaf herbicide may be used in the wheat phase of the rotation followed by a grass herbicide in the cotton crop thus creating a rotation of herbicides with different modes of action based on the type of crop. In such a cropping system, weed species have difficulties adapting because their growth

cycles are disrupted, not only because of herbicides with different modes of action being used in a particular crop, but also due to the cultural operations being used for the crop. For example, time of planting, type of tillage, and inter-row operations may be different for these different crops, thus, creating a non-friendly environment for weeds to adapt to these continuous changes in selection pressure. Further, the type of crop in the rotation also helps in reducing weed pressure. For example, wheat can be very competitive with certain weeds. A study found that one wheat plant was as competitive as 19 rigid ryegrass plants and 24 cowcockle plants.

In California, however, with huge acreages of perennial cropping systems like orchards and vineyards, it sometimes is impractical to rotate crops. Similarly, crop rotation opportunities can be limited by economics (high value crops on high value farmland) or by environmental constraints (e.g. heavy soils used for rice production in the Sacramento Valley). Non-crop areas such as roadsides, canal banks, and industrial sites also have few rotational alternatives. Therefore, in these systems, rotation of herbicides with different modes of action should be a part of the management plan to prevent the buildup of weeds that are resistant to that particular mode of action.

The selection pressure on weeds from herbicides with longer residual activities is higher than that from herbicides with shorter or no residual activities because one treatment can result in exposure of multiple weed cohorts to the herbicide. However, this does not mean that residual herbicides should be removed completely from the management program and replaced by a post-emergence herbicide-only program, as this will serve as a recipe for selection for herbicide-resistant weeds, as has been observed with glyphosate-only weed control programs. In fact, short-term residual herbicides in combination with post-emergence herbicides are being recommended for management of glyphosate-resistant common waterhemp in soybean cropping systems in Illinois. Similar strategies are being recommended for management of glyphosate-resistant palmer amaranth in cotton production systems in Arkansas, Georgia, and Tennessee.

### **Herbicide-resistant weed conclusions**

Resistance mitigation seeks to diversify weed control methods in order to delay the evolutionary process by reducing the selection pressure exerted by herbicides. Target-site resistance is conferred by an alteration causing loss of plant sensitivity to herbicides with a specific mechanism of action. It is, therefore, clear that one way of dealing with the problem is by switching to another herbicide effective on the same weed species, but having a different mechanism of action. The use of herbicide mixtures or sequences involving herbicides with different mechanisms of action can protect the herbicides and delay the evolution of resistance to each, since mutants with resistance to one herbicide would be controlled by the other herbicide and vice-versa. The recurrent use of the same herbicide mixture could theoretically select for biotypes with resistance to both herbicides (multiple resistance).

Non-target-site resistance may involve different herbicides and the enhanced expression of mechanisms that are common in plants and thus easily selected. If several herbicides share a common degradation route, such as the ubiquitous P450 monooxidation, their use will select for the same mechanism of resistance in biotypes that will be resistant to all whether these herbicides are used in mixtures or sequences with each other. Thus, combining or changing herbicides to control non-target-site-resistant biotypes becomes very difficult unless the specific mechanisms and isozymes of detoxification or sequestration in plants are known for each herbicide, which is beyond the scope of most users. Non-target-site resistance may involve the accumulation of genes contributing partial resistance levels. Such accumulation can occur via hybridization, gene duplication and/or amplification when sub lethal herbicide rates are used and individuals with partial resistance are allowed to survive.

From this discussion of resistance mechanisms in herbicide-resistant weeds, it should be clear that resistance cannot be mitigated only by switching or combining herbicides in production systems that rely solely on the intensive use of selective herbicides for weed control. Instead, herbicide resistance management requires the integrated diversification of chemical and non-chemical weed control methods to reduce selection pressure for resistant weed biotypes. Herbicides are one of the most effective tools for weed management; however, they must be used judiciously. They should be 'one of the many tools' in a weed management toolbox rather than the only tool, or else we are at risk of losing effective herbicides due to the evolution of herbicide-resistant weeds.



## HERBICIDE-RESISTANT CROPS

In recent decades, the cost of finding and developing a new herbicide active ingredient that also meets all toxicological and environmental standards has approached \$100 million. A successful alternative strategy for selective weed control in some crops has been to use techniques such as wide crossing, mutation breeding, or genetic engineering to make the crop resistant to an existing class of herbicides. Herbicide-resistant crops (HRC) were introduced in the 1980's but poor agronomic performance originally limited the usefulness of the technology. However, in the 1990's, genetically-engineered soybeans resistant to glyphosate (the active ingredient in Roundup) with little or no yield penalty were commercially released and adopted at an unprecedented rate. Since 1996, Roundup Ready™ crops have been grown on the majority of the US soybean, cotton, corn, and canola acreage and are widely used in many other countries.

### Mechanisms of resistance in crops

Herbicide resistance in crop plants is usually conferred by mechanisms similar to those that cause herbicide resistance in weeds. First, resistance can be due to lack of herbicide activity at the site of action (target site not present, target site present but herbicide binding site absent or altered, excessive amount of the target enzyme relative to the amount of herbicide). A non-target site mutation such as rapid metabolic detoxification of the herbicide is another mechanism that can allow plants to survive with little or no irreversible effects from the herbicide applications. Metabolic rates can vary among plant growth stages and environmental conditions; this is one reason why crop safety and weed control efficacy can sometimes differ dramatically in cool environments compared to more ideal growing conditions.

### Conventional vs transgenic HRC

The historical definition of herbicide-tolerant crops refers to crops that exhibit resistance through natural mechanisms that existed in the plant before introduction of the particular herbicide. However, the contemporary definition of a herbicide-tolerant crop encompasses a broader array of mechanisms, including modifications in the site-of-action of the herbicide, the metabolism and detoxification of the compound, as well as sequestration and/or limited uptake or translocation of the herbicide to its site-of-action. Such mechanisms can be exploited through classical breeding methods or biotechnology utilizing *in vitro* and molecular methods.

Problems with conventional breeding approaches have included yield-drag (reduced productivity), difficulty in finding compatible wild species with sufficient resistance, or problems with the introgression of the trait into commercial cultivars. Furthermore, even after a trait is successfully crossed into a species, multiple generations of backcrossing usually are required to restore an acceptable level of agronomic performance and homogeneity of the breeding line. These issues, combined with scientific and technological advances in molecular biology, have led to greater interest in the use of genetic engineering rather than classical breeding approaches to develop herbicide-resistant crops.

Crops created through an array of molecular and *in vitro* technologies include cultivars developed through wide-species hybridization and advanced *in vitro* technologies, such as embryo rescue, somaclonal variation, protoplast fusion, or the introduction of new genes using genetic engineering methods.

### Conventional methods

All plant species are naturally resistant to some herbicides, and susceptible to others. Conventional breeding techniques often rely on identification of potential variety parental lines through screening with the herbicide of interest. One method is to survey the crop species germplasm, as some varieties or breeding lines may display better resistance than the crop cultivars. Identifying the more resistant lines and introgressing the resistance genes through traditional crossing may be used to move the resistance genes into commercial cultivars. A second approach is to expose the crop species to sub-lethal doses of the relevant herbicide and select the best performing survivors over several generations and spray regimes.

Spontaneous mutations have also contributed to herbicide-resistant commercial cultivars. Triazine tolerant canola was first developed from a spontaneous *Brassica rapa* weed showing resistance to triazine applications in a farmer's field in Quebec. Breeders used that sole surviving plant as a parent in a conventional canola breeding program and derived the

entire series of triazine tolerant canola cultivars, which remain popular, especially in Australia, in spite of a substantial yield penalty. Although not commercially available, plant breeders have bred ALS inhibitor resistance from prickly lettuce into commercial lettuce cultivars.

Conventional breeders also use induced mutations (via ionizing radiation, mutagenic chemicals etc.) to alter the genetic makeup of a plant in the hope of creating resistance to various herbicides. The method is simple enough; take a large number of seeds, expose them to the mutagen, grow out the seeds and spray the progeny with the herbicide, select any survivors as prospective resistant mutants. Several herbicide-resistant crop cultivars were developed using mutagenesis, including wheat cultivars based on a mutagenized hard red wheat line (FS-4) with novel resistance to imidazolinone herbicides.

Biotechnological methods have also been used to create herbicide-resistant crops. In the 1980s, several groups applied the active ingredient chemical from various herbicides to plant cells growing *in vitro*, then regenerated whole plants from surviving cells. In some cases, the progeny of the regenerants showed enhanced resistance to the herbicide. However, whether any of these lines were commercialized is unclear. Similarly, somaclonal variants, plant cells growing *in vitro* that vary genotypically and/or phenotypically, have been used to select herbicide-resistant cell lines which were then regenerated into whole plants and formed the basis for new herbicide-resistant cultivars. The latter were mainly crops with resistance to Group 2 herbicides such as the Clearfield™ series of crop cultivars, including canola, sunflower, rice, corn, wheat and lentils.

### **Molecular methods**

In the late 1980s, genetic engineering methods had advanced to the point where genes for specific traits could be introduced into various crop species. Among the first practical (as opposed to experimental or marker) traits was herbicide resistance, as single genes conferring resistance to specific herbicides had been isolated, cloned and characterized.

Among the first herbicide resistance genes were those conferring resistance to glyphosate and glufosinate (the active ingredient in Basta™, Liberty™, and Rely™ herbicides). In the latter case, the genes were used initially as *in vitro* selectable markers (and are still used as such) before being developed for weed control in transgenic herbicide-resistant crops.

Around the same time period, herbicide resistance genes were being developed to provide crop resistance to other herbicides as well, such as bromoxynil (e.g., Buctril) and the sulfonylurea and imidazolinone herbicides. Among the early crop species with these genes were soybean, corn, canola, cotton and flax. In spite of considerable research and development efforts, relatively few herbicide tolerant genetically engineered crop cultivars have been released commercially. However, several of the transgenic herbicide-resistant crops that have been commercialized have proven popular with farmers.

### **Adoption of herbicide-resistant crop technology**

Herbicide-resistant crop technology, whether developed through transgenic or conventional breeding approaches, has been widely adopted by growers. Benefits to growers using herbicide-resistant crops fall into three main categories: economic savings, effective and simple weed management, and convenience and flexibility [for excellent reviews of herbicide-resistant crop issues, refer to the special issue of Pest Management Science edited by Duke and Powles (2008); a review of genetically engineered plants by Lemaux (2009); and a special issue of AgBioForum edited by Frisvold (2009)]. There currently are 11 crops with commercially available herbicide-resistant varieties (Table 4). Of these, only glyphosate- and glufosinate-resistant crops were developed using transgenic techniques while the others were selected or created using conventional breeding approaches.

Glyphosate resistance is available in six major crops (corn, soybean, cotton, canola, sugarbeet and alfalfa); these account for nearly two-thirds of the worldwide transgenic crop acreage. Soybean producers rapidly adopted glyphosate-resistance technology and grow glyphosate-resistant cultivars on over 90% of the U.S. soybean acreage (<http://www>

**TABLE 4.** CROPS WITH HERBICIDE-RESISTANT CULTIVARS COMMERCIALY AVAILABLE, PREVIOUSLY COMMERCIALIZED OR IN ADVANCED TESTING<sup>1</sup>

Crop	Previously or currently commercialized <sup>2</sup>	Advanced testing
Alfalfa	glyphosate	
Canola	glyphosate, glufosinate, bromoxynil, triazine, imidazolinones	
Corn	glyphosate, glufosinate, imidazolinone	
Cotton	glyphosate, glufosinate, bromoxynil	
Flax	sulfonylureas	
Lentil	imidazolinones	
Rice	imidazolinones	glyphosate, glufosinate
Soybean	glyphosate, glufosinate, sulfonylureas, metribuzin	synthetic auxin
Sugarbeet	glyphosate	
Sunflower	imidazolinones	sulfonylurea
Wheat	imidazolinones	

<sup>1</sup>This listing is likely incomplete in regards to non-genetically modified organism (GMO) cultivars which are not regulated or reported in the same detail as GMO-derived herbicide resistance traits.

<sup>2</sup>Does not include naturally occurring herbicide tolerance.

users of the technology in California as well. Approximately 60% of the corn and 80% of the cotton produced in California is glyphosate-resistant. Additionally, recent deregulation of the glyphosate-resistance trait in alfalfa is likely to lead to increased adoption of glyphosate-resistant alfalfa in the state. Interestingly, although imidazolinone-resistant rice has been rapidly adopted by rice growers throughout the southern U.S., those cultivars are not currently used by California rice growers. Research has been conducted on several minor crops important in California, including lettuce, potato, sugarbeet, and tomato; however, commercialization efforts on these crops have been minimal to date.

## Concerns related to herbicide-resistant crops

### *Selection for herbicide-resistant weeds*

The first portion of this chapter focused on evolution and selection of herbicide-resistant weeds and this issue is one of the largest concerns related to adoption of herbicide-resistant crops. Attributes that make herbicide-resistant crops attractive to growers (i.e. broad weed control spectrum, low application cost, fewer non-target or negative environmental effects) can lead to reliance on a single herbicide mode of action and impose tremendous selection pressure for resistant weeds. For example, although glyphosate has been used in North America since the early 1970s, glyphosate-resistant weeds were not reported until 2001, several years after the commercialization of the first Roundup Ready crop. In the years since the first report, 21 unique cases of glyphosate-resistant weeds have been reported and many appear to have been selected in glyphosate-resistant cropping systems. Among the most dramatic examples of this include glyphosate-resistant cotton, soybean, and corn systems in the Midwestern and Southeastern US afflicted with glyphosate-resistant

.ers.usda.gov/topics/farm-practices-management/biotechnology.aspx). Several South American soybean producing countries also have over 90% adoption of glyphosate-resistant soybeans. In the U.S., adoption of glyphosate-resistant corn and cotton is nearing 70% and competes primarily with other herbicide-resistant cultivars. The majority of canola grown in the U.S. and Canada is glyphosate- or glufosinate-resistant.

Adoption and deregulation of herbicide-resistant crops, primarily those derived from transgenic lines, is limited in some countries due to consumer and marketing issues. For example, few, if any transgenic herbicide-resistant crops are currently grown in the European Union countries due to strict regulations on growing, importing and labeling crops derived from genetic engineering. However, the EU has authorized import and processing of three herbicide-resistant canola cultivars for animal feed.

### **Herbicide-resistant crops in California**

Herbicide-resistant crop use is somewhat limited in California because of the state's focus on specialty crops as well as a yield drag associated with early glyphosate or glufosinate tolerant cotton cultivars. Most herbicide-resistant crop development efforts have focused on large acreage, commodity crops including cereal grains, oilseed crops, cotton, and alfalfa and these are also the primary

pigweeds (*Amaranthus palmeri*, *A. rudis*, *A. tuberculatus*) and horseweed/marestail to the point of almost total failure in some regions [see Nandula 2010 and other references for more detail]. In these cases, as in other cases of evolved herbicide resistance, selection is driven by the lack of diversity in the weed control program and nearly total reliance on one method of weed control. Just like in non-HRC crops, resistance management in HRC should include an integrated weed management approach that utilizes multiple herbicide modes of action as well as appropriate non-chemical strategies. HRC do not preclude the use of conventional herbicides and, if used as part of an integrated strategy should actually increase the number of available weed management options for growers.

#### *Managing herbicide-resistant crop volunteers*

Herbicide-resistant crop volunteers that emerge in subsequent crops can be a significant agronomic concern and may require alternate management practices compared to susceptible crop volunteers. Herbicide-resistant crop volunteers may become more problematic and difficult to control if another crop resistant to the same herbicide is planted in rotation. For example, volunteer soybean in cotton became a problem in the southeastern US with the commercialization of glyphosate-resistant soybean and cotton. Similar problems with glyphosate-resistant volunteer corn have been observed in corn-soybean cropping systems throughout the US and Canada after widespread adoption of glyphosate-resistant cultivars in both crops and in corn-cotton rotations in California. Herbicide-resistant crop volunteers can also contribute to pollen and seed-mediated gene flow by dissemination of transgenes via hybridization and seed dispersal. The problem of herbicide-resistant crop volunteers usually is managed like other herbicide-resistant weeds, primarily through tillage or the use of herbicides with a different mode of action from the one used in the earlier rotation. In fact, using an herbicide with another mode of action to control volunteers may mitigate or delay evolution of herbicide-resistant weeds by ensuring that farmers include herbicides from several chemical families in their weed management program.

#### *Effects on rotational crops*

Herbicides differ in both their residual activity and recommended plant-back intervals for different crops. One of the benefits to farmers using herbicide-resistant crops is a decrease in the rotation time between the harvest of one crop and the planting of the next because a sensitive crop cannot be rotated into a field until a residual herbicide has dissipated to the point that the rotational crop will not be affected. Herbicide-resistant crops, which share an herbicide resistance trait, would allow farmers to rotate crops promptly; however, this practice is likely to encourage mono-herbicide application which is detrimental to resistance management.

#### *Gene flow*

Gene flow is important in the maintenance of genetic variation in populations as well as in the spread of new traits among populations and across species boundaries. Gene flow can occur by pollen movement or by direct movement of seed or in some cases by vegetative propagules, but within an agronomic system these pathways often are linked. Global expansion in cultivation of herbicide-resistant crops has increased concerns regarding pollen and seed-mediated gene flow from herbicide-resistant crops to conventional and organic crops. Although gene flow is not unique to herbicide-resistant crops, it has been suggested consistently and repeatedly as an environmental concern. The primary areas of concern are that unexpected gene flow could cause changes in food safety, harm markets, and lead to changes in biodiversity of agricultural or natural areas.

#### *Pollen-mediated gene flow*

Pollen-mediated gene flow is transfer of genetic information between plant populations resulting from cross-pollination. Gene flow between populations of the same species is known as *intra-specific* gene flow whereas gene flow among different species (e.g. between domesticated crops and wild relatives) is known as *inter-specific* gene flow. A number of factors determine the likelihood and extent of intra-specific pollen-mediated gene flow among plant populations. These include the reproductive biology of a species or cultivar within a species, flowering phenology, sexual compatibility, pollen load, and environmental conditions at specific vegetative or reproductive stages of plant development, spatial and temporal distribution of pollen donors and recipient plants and also size of the pollen donor and recipient plant populations.



A risk associated with the use of transgenic herbicide-resistant crops is that transgenes could be transferred to wild or weedy relatives by inter-specific hybridization. Hybridization between crops and wild relatives has been documented to some extent in 12 out of 13 major food crops including wheat, rice, sorghum, sugarbeet, soybean, corn, barley, and canola. In several instances (sugarbeet, rice, canola), substantial levels of gene flow to wild relatives was observed under conditions commonly encountered in agricultural settings. Inter-specific hybridization depends first on the presence of compatible wild relatives. In the U.S., there is a low risk of transgene movement from herbicide-resistant soybean and corn cultivars to wild relatives because few relatives are present. However, other crops in the U.S. that do have wild and weedy relatives with which gene flow is possible include canola, alfalfa, sunflower, creeping bentgrass, wheat, sugarbeet and rice. Consideration of crop-to-wild gene flow is essential during ecological risk assessments conducted before the commercialization of herbicide-resistant crops.

### *Seed-mediated gene flow*

Seed-mediated gene flow primarily occurs through seed dispersal within and among agricultural fields but may also be facilitated by seed spill during transport, admixture of seed used for planting and by co-mingling of seeds within the seed handling system. Seed mediated gene flow may occur by natural dispersal mechanisms via animals, wind or water or by human actions such as tillage and transport. Unlike natural seed-mediated gene flow, seed movement by humans results in a limitless dispersal capability. Due to the 'permeable' nature of the supply chain, seed-mediated gene flow can occur at most stages of production. Although seed-mediated gene flow is a large source of transgene input into the environment and the agricultural supply chain, it has received relatively little attention in the scientific literature compared to pollen-mediated gene flow from herbicide-resistant crops.

### *Environmental issues*

There are environmental issues related to herbicide-resistant crop use, which, although not unique to genetically engineered cultivars, are still relevant. Herbicides are widely used at present, and most areas where farmers will use herbicide-resistant crops are already treated with herbicides as part of a weed control program. The herbicides for which herbicide-resistant crops are being developed are, in general, low-use-rate, low-mammalian-toxicity herbicides with short soil half-lives; therefore, the use of these herbicide-resistant crops should result in lower environmental impact than occurs at present with higher impact herbicides.

### *Herbicide use*

The existence of herbicide-resistant crops does not necessarily translate into an increased use of herbicides. If industry succeeds in making herbicide-resistant crops cost-effective, the herbicides to which tolerance is being engineered will almost certainly increase in sales. An increase in sales does not necessarily mean that farmers are applying more herbicide per acre, but may simply mean that the acreage being treated using these herbicides has been increasing and/or that the older herbicides are being replaced by those used with the transgenic cultivars. While herbicide-resistant crops do encourage continued use of herbicides, they also offer farmers alternatives at a time when the development and registration of new herbicides or other effective weed management strategies has been slowed by market, economic, and regulatory issues.

### *Water and environmental quality*

Most herbicide-resistant crops are based on glyphosate, glufosinate, imidazolinone, and sulfonylurea herbicides. The specific herbicides and application rates used in these crops usually have acceptable persistence in soil, low mammalian toxicity, and fewer issues with leaching than compounds such as simazine and diuron which are commonly found in California ground water. Rotation of cropping systems with herbicide-resistant crops and non-engineered varieties of the same crop species should encourage the rotation of herbicides, thereby reducing environmental buildup.

Interestingly, the selective, in-season weed control allowed by herbicide-resistant crops greatly facilitated the adoption of minimum- and no-tillage farming systems. Reductions in tillage operations conducted solely for weed control has had many environmental benefits including reduced soil erosion, greater water infiltration, and less pesticide and fertilizer contamination in surface waters [see Duke and Powles 2009 and associated references for more detail].

*Economic issues*

The additional cost of herbicide-resistant crops, coupled with the cost of the herbicides, imposes some additional economic burdens on farmers; however, indiscriminate use of herbicides will never be cost-effective, nor would it be permissible based on U.S. Environmental Protection Agency (EPA) policy. In order to be adopted in the marketplace, herbicide-resistant crops must lead to weed control strategies that provide an economic and/or managerial benefit to growers and be acceptable to the consumers of the end products. Attaining this goal is time-consuming; estimates of the time required for the identification and introduction of a tolerance trait are six years or more. Acceptance and establishment of the HRC cultivars in the marketplace could take several more years.

*Effects on the herbicide industry*

Cropping systems developed to rely on only a few nonselective herbicides can be expected to affect the development and commercialization of new alternative herbicides. The development of herbicide-resistant crops has already led to a global restructuring of private-sector agrochemical research, development, and marketing systems. Certainly, in the near term, development activity will continue to focus on herbicide-resistant crop strategies, but the effect of this on the development of other agrichemical approaches is not known.

Perversely, the recent agrichemical research focus on herbicide resistance technology in major crops has had a large, negative impact on weed control options for minor acreage crops. Most herbicides used in small acreage specialty crops were originally developed for large-acreage crops; thus reduced herbicide-discovery efforts in major crops have also led to fewer new registrations in minor crops. Herbicide resistant cultivars would be extremely valuable for many specialty crops that currently rely on a limited number of registered herbicides and increasingly expensive hand labor for weed control. However, costs to develop the technology for relatively minor acreage crops with limited market potential is the primary factor limiting herbicide resistant crop development by private industry or public institutions.

Some restructuring of agrichemical companies toward a more biological-, less chemical-based, focus is already being seen in the marketplace; the long-term effects of these changes on the development of classical chemical approaches to weed protection can only be seen over time. Economic assessments of the advantages of herbicide-resistant crop systems have been and will be performed and those studies will weigh heavily in decisions on the advisability of these particular approaches to weed management in the future.

*Labeling*

Labeling foods as being derived from GMO crops has been a controversial issue. The call for such labeling has been resisted in the US and Canada because mandatory labels in those countries are based on the composition and features of the final food, rather than on the particular method of breeding or processing. Not least of the problems is that there is no standard definition of a “GMO” food. Every jurisdiction, from the US to Canada to the European Union (EU), has different definitions of what would need to be labeled. For example, the EU already has a process trigger for food labels, such that any food made ‘with’ GMOs requires a label. However, foods made ‘from’ GMOs do not. To avoid any confusion that may arise in distinguishing between food made ‘from’ GMOs and those made ‘with’ GMOs, the US and Canada use an objective compositional trigger for mandatory labels, so only foods with a measurable nutritional compositional change, or changes to levels of substances normally present (including allergens or other antinutritional substances) are required to carry labels.

Similarly, even those advocating mandatory process based labels are divided on what constitutes a GMO food for labeling. Some advocate that only those food products with ‘foreign’ genes and proteins need be labeled while others say all foods derived from a GMO should be labeled. Clearly, a tomato carrying a unique gene and protein from a fish would be labeled according to all stated criteria, but what about a tomato with a gene and protein from a different tomato? What about rice carrying a disease resistance gene from a distant relative? This has actually occurred, with the rice bacterial disease resistance gene called Xa21 having been transferred—using both conventional breeding to develop one new variety, and a second developed using biotechnology—from a non-cultivated rice

relative into commercial rice cultivars. Both new varieties carry the same genes and produce the same proteins, and are similarly newly resistant to the bacterial infection, yet only the GM-derived line would require labeling under current definitions.

### **Export markets**

The term “adventitious presence” refers to the unintentional and incidental commingling of trace amounts of one type of seed, grain or food product with another. When used in relation to herbicide-resistant crops, the term describes the inadvertent presence of herbicide-resistant crop seeds or other material in conventional or organic crop seeds. Low levels of impurities are inherent in commodity crops due to the nature of the supply chain; herbicide-resistant crops are grown in close proximity to other crops and common equipment is frequently used to plant, harvest, transport and store grains.

Variation in the tolerance and traceability requirements for adventitious presence differs greatly among countries and impacts on international trade. In recent years, the differential treatment of herbicide-resistant crops between international trading partners has caused friction, with commodity grain shipments carrying trace amounts of genetically engineered material occasionally being refused at the intended receiving port. If an herbicide-resistant crop is approved and commercialized in one country without being approved in the market countries, detection of even a tiny amount in a shipment—even below one seed in ten thousand, the technical limit of detection—can be used to refuse the entire load. Such refusals have occurred for several commodities in international markets, including corn, rice, soybean and, recently, flax.

A full discussion of the controversies surrounding labeling of GMO crops (not just herbicide-resistant cultivars) and acceptance in domestic and export markets is beyond the scope of this book. However, these issues are likely to remain significant factors affecting the development, adoption, and market acceptance of agricultural products developed using biotechnology techniques including future herbicide-resistant crop cultivars.

### **Conclusions**

Differential tolerance or resistance to herbicides among plant populations and biotypes presents both tremendous opportunities and challenges in weed management. The discovery of herbicide tolerance in crop plants formed the basis for modern chemical weed control and has led to large gains in crop yields and production efficiency. Multiple herbicides from various chemical families offer varying levels of selective and non-selective weed control from preemergence and post emergence applications and are currently used on the vast majority of crop production acres in most modern agricultural systems. Advances in both conventional and transgenic plant breeding techniques, offer new opportunities for selective weed control by allowing the application of highly effective, reduced toxicity, broad spectrum herbicides to herbicide-resistant varieties of important field crops. However, although widely used in some cropping systems, herbicide-resistant crops are not without controversy and concerns about the effects on herbicide use, gene flow, and export market acceptance remain in some cases.

Although herbicides have undeniably and dramatically changed agricultural productivity, these highly effective and selective weed management tools impose high selection pressure on weed populations and can lead to weed communities composed of tolerant or resistant species. Herbicide-resistant weeds have become a serious management challenge in many cropping systems around the world and can lead to yield reductions, increased weed control efforts and costs, and more complicated management decisions. Many weedy species with resistance to one or more herbicide classes have been reported; however, certain genera appear to be more prone to resistance. Target site or non-target site based resistance to an herbicide or to multiple herbicide classes has provided insights into the physiology, genetic diversity, and evolution of non-crop plants. This basic research is leading to the development of better weed management strategies on an applied basis and, in recent years, weed research and education efforts in many states and counties have focused on using best management practices to prevent or manage herbicide-resistant weed populations while stewarding the available herbicides and herbicide-resistant crop technologies.

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