

# Herbicide Tolerant Crops and Weed Management

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**Abstract:** *The development of weed resistances to many selective herbicides and the prohibitive expense and difficulty associated with the development of new herbicides, a need has arisen to seek alternatives to address these challenges. Along with the efforts to discover new herbicide target sites in plants, biotechnology is making major contributions in broadening crop selectivity to the already existing and effective herbicides. Further efforts to create more herbicide tolerant crops are needed to ensure more economical crop production and safeguard environmental quality by reducing the demand for and the number of selective weed killing chemicals required for economical crop protection.*

**Keywords:** *Herbicide, weed, disease, resistance*

## 1. Introduction

Farmer must control weeds that compete with their crops for water, nutrients and sunlight. A number of options for minimizing the impact of weeds on crop productivity are available to growers: one option is the application of herbicides. Herbicide treatment in crop plantings has allowed economically viable weed control [1] and increased productivity. The most preferred herbicides today are those that combine weed killing potency with low- or no environmental persistence. However, the very effective broad spectrum herbicides available also lack selectivity, thus limiting their use in some cropping operations. On the other hand, the continuous use of the few available selective herbicides is speeding up the development of herbicide resistance in weeds; hence making it difficult to achieve effective control in some crops [15].

Virtually all crops have some degree of innate tolerance to some herbicides but not others. In the 1940's, this selective tolerance started to be used to control weeds in corn fields. Today growers all over the world minimize the negative impact of weeds by using herbicides and herbicide tolerant crops, including all of the major commodity crops, as well as small acreage horticultural crops, such as vegetables. An ancillary benefit of herbicides is the impact their use has on soil erosion. The use of herbicides and herbicide tolerant crops enables growers to use minimal tillage or no-till techniques, which significantly decreases the impact of agriculture on soil erosion.

Since the 1990s herbicide tolerant crops, developed with modern biotechnology, have significantly increased growers' profits by decreasing their input costs, increasing yields, or both, in some cases as well as save soil from erosion. As a result of these benefits, the rate of adoption of biotech herbicide tolerant crops has been quite rapid. For example in Argentina glyphosate-tolerant soybeans were planted on over 98% of soybean acres within 5 years of introduction.

Two approaches can be pursued to achieve this goal. The first is the design of specific chemicals with broad selectivity for crops. This approach, however, is expensive and the products thereof may be uneconomical for use by growers, not to mention that it is also away to increase the already growing chemical load to the environment. According to Gressel (2002), it has become increasingly difficult to discover new herbicides and even harder to come up with one that has a novel mode of action. In the 1940s, only about 500 compounds needed to be screened to select a potential herbicide [17].

By 1989, it was estimated that the discovery of one selective herbicide involved the screening of more than 30 000 compounds and after identification these compounds had to be further modified to improve their toxicity to target weeds and their rapid metabolism in crops (Parry, 1989). In addition "chemical handles" have to be designed to aid the rapid delivery of new chemicals into the target weed plant systems (Owen and DeBoer, 1995). Today the discovery of a potential herbicide requires the screening of nearly 500 000 compounds (Tan et al., 2005). The second and more popular approach to crop herbicide selectivity is the development of crop cultivars with tolerance to the already existing effective broad spectrum herbicides so as to expand the crop options in which they can be used. Two methods can be used to develop crops with resistance to herbicides. Conventional plant breeding utilizing lines that are known to be tolerant to specific herbicides is one approach that could confer resistance to susceptible crops from closely related species. However, this approach has limitations in that naturally herbicide resistant plants are found more among weed species than in crops. Also, conventional plant breeding takes a long time to produce a single useful line. A faster approach is the use of biotechnology techniques such as *in vitro* cell culture, mutagenesis [9] and selection in physiologically inhibitory concentrations of herbicides (also referred to as brute force selection) or genetic transformation of already existing crop cultivars with genes that confer resistance to

herbicides. The purpose of this communication is to summarize the results of studies towards the development of crop herbicide selectivity using biotechnology techniques and highlight some of the crop products that have been developed using these techniques.

## 2. Resistance Definitions and Development

Herbicide resistance is the inherited ability of a plant to survive and reproduce following selection with a dose of herbicide normally lethal to the wild type of the plant. The development of herbicide resistance in weeds is an evolutionary process. Weed populations are extremely diverse genetically. In some cases, the genetic variation within weed populations includes the inherent abilities to resist some herbicides. However, the frequency of such variation in a normal weed population is very low. However, if an herbicide is applied repeatedly on those populations (or herbicides from the same herbicide group are applied), the entire picture can change. As the majority of the susceptible biotypes are controlled after repeated applications, the few resistant biotypes are provided with a unique opportunity to proliferate. Therefore, the use of an herbicide or herbicides from the same herbicide group continuously for many years can drastically decrease the number of susceptible biotypes within the natural weed population and dramatically increase the number of resistant biotypes. In response to widespread use of a particular family of herbicides, weed populations can change in genetic composition such that the frequency of resistance gene(s) and resistant individuals increases. Thus, weed populations become adapted to the intense selection imposed by herbicides [2].

## 3. What are some of the advantages of GM foods?

The world population has topped 6 billion people and is predicted to double in the next 50 years. Ensuring an adequate food supply for this booming population is going to be a major challenge in the years to come. GM foods promise to meet this need in a number of ways:

- **Pest resistance** Crop losses from insect pests can be staggering, resulting in devastating financial loss for farmers and starvation in developing countries. Farmers typically use many tons of chemical pesticides annually. Consumers do not wish to eat food that has been treated with pesticides because of potential health hazards, and run-off of agricultural wastes from excessive use of pesticides and fertilizers can poison the water supply and cause harm to the environment. Growing GM foods such as B.t. corn can help eliminate the application of chemical pesticides and reduce the cost of bringing a crop to market.
- **Herbicide tolerance** For some crops, it is not cost-effective to remove weeds by physical means such as tilling, so farmers will often spray large quantities of different herbicides (weed-killer) to destroy weeds, a time-consuming and expensive process, that requires care so that the herbicide doesn't harm the crop plant or the environment.

Crop plants genetically-engineered to be resistant to one very powerful herbicide could help prevent environmental damage by reducing the amount of herbicides needed. For example, Monsanto has created a strain of soybeans genetically modified to be not affected by their herbicide product Roundup®.

A farmer grows these soybeans which then only require one application of weed-killer instead of multiple applications, reducing production cost and limiting the dangers of agricultural waste run-off.

- **Disease resistance** There are many viruses, fungi and bacteria that cause plant diseases. Plant biologists are working to create plants with genetically-engineered resistance to these diseases.
- **Cold tolerance** Unexpected frost can destroy sensitive seedlings. An antifreeze gene from cold water fish has been introduced into plants such as tobacco and potato. With this antifreeze gene, these plants are able to tolerate cold temperatures
- **Drought tolerance/salinity tolerance** As the world population grows and more land is utilized for housing instead of food production, farmers will need to grow crops in locations previously unsuited for plant cultivation.
- **Pharmaceuticals** Medicines and vaccines often are costly to produce and sometimes require special storage conditions not readily available in third world countries. Researchers are working to develop edible vaccines in tomatoes and potatoes. These vaccines will be much easier to ship, store and administer than traditional injectable vaccines.

## 4. Cell culture and Selection

Plant tissue culture represents the simplest of the biotechnologies available to plant scientists today. The realization that certain *in vitro* conditions could induce heritable changes, called somatic clonal variations, in the genomes of plant cells opened an avenue for the selection of various desirable traits from *in vitro* cultures, including herbicide resistance (Maliga, 1984). Using cell culture procedures, BASF Inc. produced a corn hybrid (DK404SR) that is resistant to the sulfonylurea herbicide, Sethoxidim. Cell culture [3] under lethal concentrations of certain herbicides also results in gene amplification in surviving cells that leads to resistance through the overproduction of enzymes targeted by herbicides. A petunia cell line with resistance to glyphosate was selected in this manner and plants regenerated from it survived lethal levels of glyphosate (Steinrücken et al., 1986). This resistance was found to be due to amplification of the gene encoding 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase that caused its overproduction in the cells (Steinrücken et al., 1986). Other *in vitro* cell selection studies have developed resistance to paraquat in tomato cells (Thomas and Pratt, 1982), resistance to glyphosate in carrot and groundnut cells [5] and resistance to a Protoporphyrinogen oxidase [7] (PPO) inhibitor in soybean cells [6] however, no viable plant regeneration was reported in these studies.

## 5. Mutagenesis

Chemical or physical mutagenesis of seed, microspores or pollen followed by selection under herbicide selective pressure has also been utilized to develop crop resistance to herbicides. The most common mutagen employed is ethyl methanesulfonate (EMS), which is efficient in producing chloroplast mutants [4]. In this method, seeds or pollen are treated with EMS then grown either *in vitro* or *in vivo* in the presence of an herbicide. Surviving plants are selected and grown to maturity to provide seed that is used for further screening with herbicides. Utilizing this method, [8] developed 21 Brazilian rice lines that were resistant to glyphosate. [10] Produced atrazine resistant *Solanum melongena* plants by mutagenizing seeds followed by germination and *in vitro* regeneration of plants from the resultant seedling cotyledons. This change prevents the binding of the herbicide to the enzyme active site, thus maintaining normal enzyme function. Recently, [11] reported the production of atrazine-resistant pepper (*Capsicum annuum*) plants regenerated from three-week old seedling cotyledons obtained from EMS treated seeds. They also observed maternal inheritance of the atrazine resistance trait.

## 6. Genetic Transformation

A number of GM crops expressing various traits have been commercialized and several are at various stages of development (TRANSGEN 2005). Herbicide tolerance is the most common trait in commercial transgenic crops, being part of 82% of all transgenic crops in the year 2003 [12]. Several techniques are now available for the transfer of genes (genetic engineering) into crop plants, including *Agrobacterium*-mediated gene transfer, micro-projectile (or particle) bombardment, polyethylene glycol-mediated DNA transfer and cell (protoplast) electroporation. The most commonly employed techniques in developing herbicide resistant crops are the *Agrobacterium* and the particle bombardment methods, respectively (Tsaftaris, 1996). Herbicide tolerance via genetic transformation can be conferred by one or a combination of these four mechanisms [18]:

Introduction of a gene(s) coding for an herbicide detoxifying enzyme(s);

Introduction of gene(s) coding for a herbicide insensitive form of a normal functioning enzyme or over expression of the genes coding for a herbicide target enzyme such that the normal metabolic functioning is still achieved in the plant even though some of the enzyme is inhibited;

Modification of the herbicide target enzyme in such a way that the herbicide molecule does not bind to it and;

The more recently described engineering of active herbicide efflux from plant cells. These mechanisms have variously been explored in the production of crops that are resistant/ tolerant to various herbicide classes as discussed below.

## 7. Types of Resistance

In some cases, resistant weeds can also survive the application of herbicides other than the herbicide to which

they have developed resistance (i.e., the selecting herbicide). In such cases, resistant weeds are considered to have cross- or multiple resistance. Cross-resistance occurs when one resistance mechanism (e.g., enhanced herbicide metabolism) allows the plant to withstand herbicides from different chemical classes. However, when a plant has multiple resistance it possesses two or more distinct resistance mechanisms (e.g., two or more altered sites of action), which allow the plant to resist herbicides from different chemical classes (Hall et al., 1994). For example, a population of smooth pigweed (*Amaranthus hybridus*) from Illinois has resistance to atrazine, a photosynthesis-inhibiting herbicide; multiple resistance to primisulfuron (Beacon), a sulfonylurea herbicide; and cross-resistance to imazamox (Raptor), an imidazolinone herbicide [16]. On the contrary, in some weeds, resistance to one herbicide results in increased susceptibility to another herbicide or other abiotic factors, such as standard cultivation practices, and/or biotic factors, such as effects of insect pests or infection by viruses and fungi. This phenomenon is known as negative cross-resistance (Gressel and Segel, 1990) and could be exploited in some resistant weed species. For example, Salhoff and Marton [19] reported that triazine resistant *Kochi* biotypes from Idaho were more sensitive to 2, 4-D than susceptible biotypes.

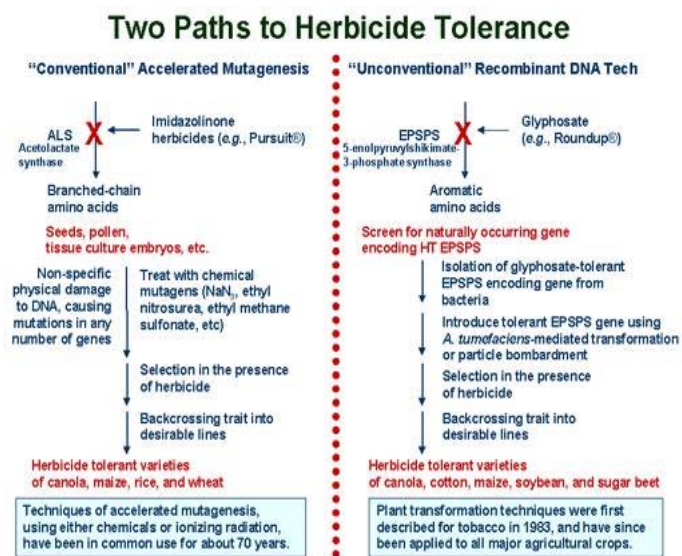


Figure 1: Two paths to Herbicide Tolerance

## 8. Current Herbicide Technologies

Besides glyphosate, most current herbicides used for weed management in corn, soybean and cotton are selective and typically used in mixtures to control a broad spectrum of weed species.

### Glyphosate

Glyphosate is a non selective, broad-spectrum foliar herbicide with no soil residual activity that has been used for >30 years to manage annual, perennial, and biennial herbaceous grass, sedge, and broadleaf weeds as well as unwanted woody brush and trees. Glyphosate is



labeled to control over 300 weed species. Many glyphosate formulations and salts are commercially available; the most common salts are the monopotassium and isopropylamine. The type and amount of adjuvant included in the various formulations differ greatly and strongly influence weed control. Glyphosate strongly competes with the substrate phosphoenolpyruvate (PEP) at the EPSPS enzyme-binding site in the chloroplast, resulting in the inhibition of the shikimate pathway.

Products of the shikimate pathway include the essential aromatic amino acids tryptophan, tyrosine, and phenylalanine and other important plant metabolic products. Favourable physicochemical characteristics, low cost, tight soil sorption, application flexibility, low mammalian toxicity, and availability of GR crops have helped make glyphosate the most widely used herbicide in the world.<sup>32</sup> A key advantage for glyphosate has been the consistent control of weeds almost without regard to size.

### Glufosinate

Glufosinate is a non-selective, broad-spectrum foliar herbicide with no soil residual activity that inhibits glutamine synthetase [GS; EC 6.3.1.2], an enzyme that catalyzes the conversion of glutamate plus ammonium to glutamine as part of nitrogen metabolism.<sup>31</sup> Glufosinate is faster acting and controls key broadleaf weeds such as morning glories (*Ipomoea* spp.), hemp sesbania (*Sesbania herbacea* (P. Mill.) McVaugh), Pennsylvania smartweed (*Polygonum pennsylvanicum* L.), and yellow nutsedge (*Cyperus esculentus* L.) better than glyphosate. However, glufosinate is used at higher rates and has historically been more expensive than glyphosate. Cost and more restrictive application timing relative to weed size are probably its greatest disadvantages compared to glyphosate.

### Synthetic Auxins

Synthetic auxin herbicides act as auxin agonists by mimicking the plant growth hormone indole-3-acetic acid (IAA), disrupting growth and development processes, and eventually causing plant death, particularly in broadleaf species.<sup>31</sup> Growers have used auxin herbicides widely for over 60 years as selective herbicides in monocotyledonous crops. Auxins control a broad spectrum of broadleaf weeds, including key weeds that have evolved resistance to glyphosate. Some synthetic auxins such as dicamba have fair soil residual activity with a half-life from 7 to 21 days. Relatively few weeds have evolved resistance to auxin herbicides, which is noteworthy considering their long-term and widespread use. For example, only six weed species have evolved resistance to dicamba after 50 years of widespread use in cereal and non-crop environments.

### HPPD Inhibitors

The enzyme 4-hydroxyphenyl pyruvate dioxygenase [HPPD; EC 1.13.11.27] converts 4-hydroxyphenyl pyruvate to homogentisate, a key step in plastoquinone biosynthesis. This is the most recently discovered herbicide MOA, and active analogue testing continues to

generate new products.<sup>37</sup> Inhibition of HPPD causes bleaching symptoms on new growth by indirectly inhibiting carotenoid synthesis due to the requirement of plastoquinone as cofactor of phytyl desaturase [PDS; EC 1.14.99].<sup>38</sup> Visible injury depends on carotenoid turnover and thus is slower to appear on older tissues than young leaves.<sup>31</sup> HPPD-inhibiting herbicides control a number of important weed species and may have soil residual activity, and no weeds have been formally reported to be resistant to this MOA yet. Corn is naturally tolerant to key HPPD herbicides, but soybeans and cotton are generally sensitive.

### ALS Inhibitors

Herbicides that inhibit acetolactate synthase (ALS; EC 2.2.1.6), also known as aceto-hydroxyacid synthase (AHAS), were discovered in the mid-1970s and are still widely used.<sup>39,40</sup> The ALS enzyme is a key step in the biosynthesis of the essential branched-chain amino acids valine, leucine, and isoleucine. ALS is a nuclear encoded enzyme that moves to the chloroplast via a transit peptide. More than 50 different ALS-inhibiting herbicides from five different chemical classes (sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidinylthiobenzoates, and sulfonylamino-carbonyl-triazolinones) are commercially available. The characteristics of ALS herbicides vary in their soil residual properties, crop response, and types of weeds that are effectively controlled.

### PPO Inhibitors

Protoporphyrinogen oxidase (PPO; EC 1.3.3.4) is an essential enzyme that catalyzes the last common step in the biosynthesis of heme and ultimately chlorophyll by the oxidation of protoporphyrinogen IX to protoporphyrin IX. PPO-inhibiting herbicides cause the accumulation of protoporphyrinogen IX, which is photoactive, and exposure to light causes the formation of singlet oxygen and other oxidative chemicals that cause rapid burning and desiccation of leaf tissue. The soil residual and fast action characteristics of PPO herbicides complement the lack of soil residual and the slow activity of glyphosate.

### ACCase Inhibitors

Acetyl coenzyme A carboxylase [ACCase; EC 6.4.1.2] is the first step of fatty acid synthesis and catalyzes the adenosine triphosphate (ATP)-dependent carboxylation of malonyl-CoA to form acetyl-CoA in the cytoplasm, chloroplasts, mitochondria, and peroxisomes of cells.<sup>43</sup> ACCase-inhibiting herbicides generally inhibit the ACCase activity of monocot species and not dicots. The three chemical classes of ACCase inhibitors are cyclohexanediones (DIMs) (e.g., sethoxydim), aryloxyphenoxypropionates (FOPs) (e.g., quizalofop), and phenylpyrazolines (DENs) (e.g., pinoxaden). The ability to use ACCase herbicides selectively in corn would be useful, but the tendency of weeds to evolve resistance to this herbicide class would limit its utility to being part of a weed management system.

## 9. Summary and Conclusion

Following the development of weed resistances to many selective herbicides and the prohibitive expense and difficulty associated with the development of new herbicides, a need has arisen to seek alternatives to address these challenges. Along with the efforts to discover new herbicide target sites in plants, biotechnology is making major contributions in broadening crop selectivity to the already existing and effective herbicides. Further efforts to create more herbicide-tolerant crops are needed to ensure more economical crop production and safeguard environmental quality by reducing the demand for and the number of selective weed killing chemicals required for economical crop protection.

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