



Glufosinate Mode-of Action:

Glufosinate (also known as phosphinothricin) is a non-selective ammonia assimilation inhibitor isolated from the bacteria *Streptomyces viridichromogenes*. This inhibitor is a phosphinic analog of glutamate and occurs naturally as one component of a small herbicidal tripeptide called bialaphos (Boger and Sandyman, 1990). The commercial product is the isolated herbicidal peptide component; this product is currently produced by chemical synthesis. Glufosinate is an inhibitor of the enzyme glutamine synthetase (GS) (reviewed by Ray, 1989, and Lea, 1991). At this site-of-action, glufosinate competes with glutamate binding at the GS catalytic domain (figure 2). Once bound to GS, glufosinate is phosphorylated to form a transition-state mimic. This mimic is then irreversibly bound to GS, resulting in deactivation of the enzyme (Lea and Ridley, 1989). The herbicidal result of GS inhibition is the rapid accumulation of ammonia in plant chloroplasts. Ammonia is a known uncoupler of photosynthetic electron transport in plant cells. Ammonia accumulation can occur within 1 hour of glufosinate treatment, with initiation of photosynthetic inhibition following in as little as 4 hours; complete photosynthetic inhibition can occur within 8 hours; free ammonia can increase within the treated cell by 10-fold within this period. This activity is light dependent, as are glufosinate induced visible symptoms of herbicide injury. Light dependency is likely the result of the inhibited ammonia reassimilation from photorespiration-produced ammonia or light-dependent nitrate reduction.

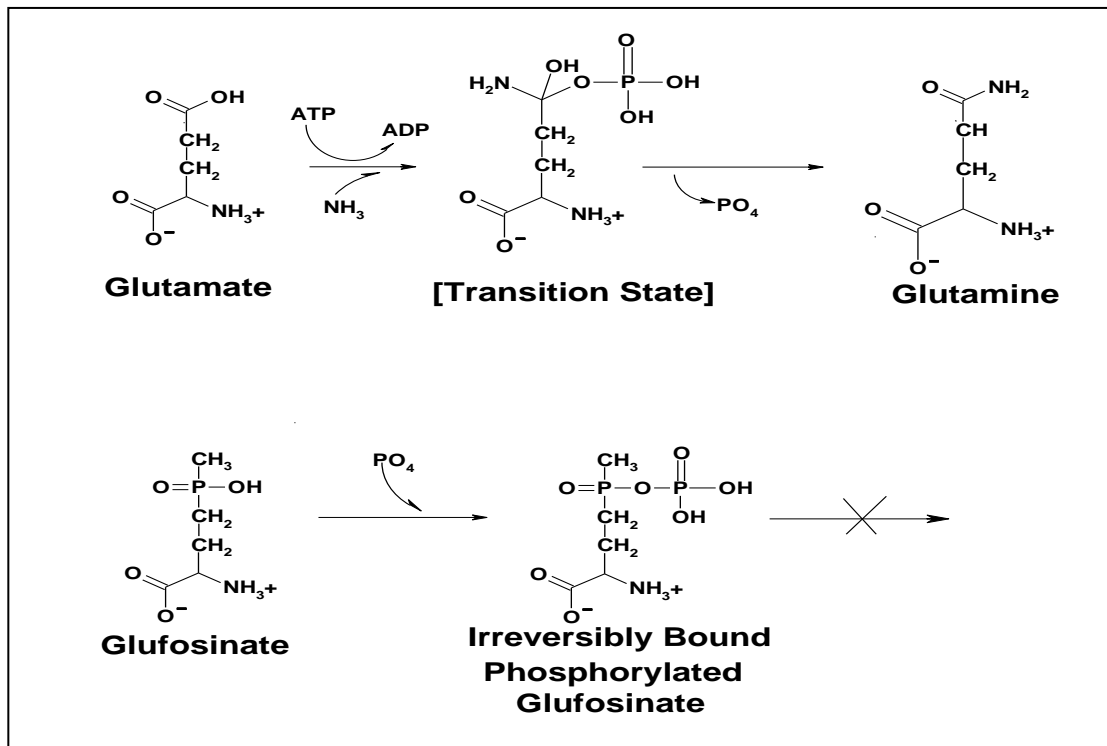


Figure 2: Incorporation of ammonia into glutamine by glutamine synthetase (GS). Glufosinate is a transition-state mimic of glutamate and binds irreversibly to GS

There is strong evidence that the inhibition of photosynthesis by glufosinate is not due to ammonia accumulation alone (Lea P.J. 1991; Gonzalez-Moro *et al.*, 1995). A second mechanism for photosynthetic inhibition results from the depletion of amino acids as a downstream effect of GS inhibition; this action may be the primary cause of glufosinate herbicidal activity (figure 3). In this scenario, amino acid depletion due to GS inhibition results in a depletion of amino ( $\text{NH}_2$ ) donors for the glycolate pathway during photorespiration. The glycolate pathway mediates the oxidation of glycolate to produce glyoxylate for the ultimate production of the amino acid glycine. Since the conversion of glyoxylate to glycine is prevented by the depletion of amino donors, several metabolic intermediates accumulate, including phosphoglycolate, glycolate and glyoxylate. Several studies (most recently, Gonzalez-Moro *et al.*, 1995) have shown that glyoxylate inhibits photosynthesis by preventing the activation of RuBP, a key enzyme involved in photosynthetic  $\text{CO}_2$  fixation. Inhibition of photosynthesis results in membrane damage, chlorophyll bleaching, and ultimately in tissue necrosis. Glufosinate induced plant necrosis normally occurs in 1 to 5 days.

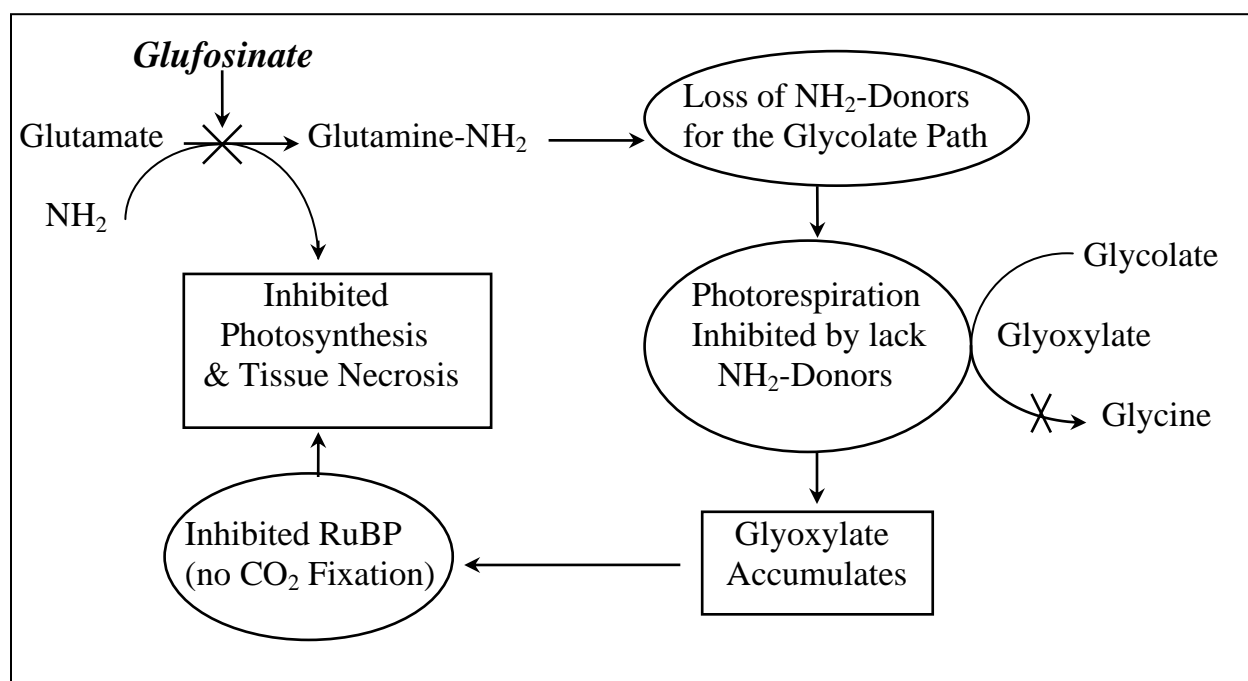


Figure 3: Glufosinate Mode-of-Action

Activity:

Glufosinate is a non-selective herbicide used at 1 to 1.5 lb./A. This inhibitor has non-systemic contact activity, is not active by root uptake, and has minimal translocation within the whole plant. Glufosinate is rapidly degraded in soil.

Glufosinate Resistant Crops:

Several crops (corn, soybeans and canola) have been genetically engineered to possess resistance to glufosinate. The glufosinate resistance gene, (called *bar* for bialaphos resistance)

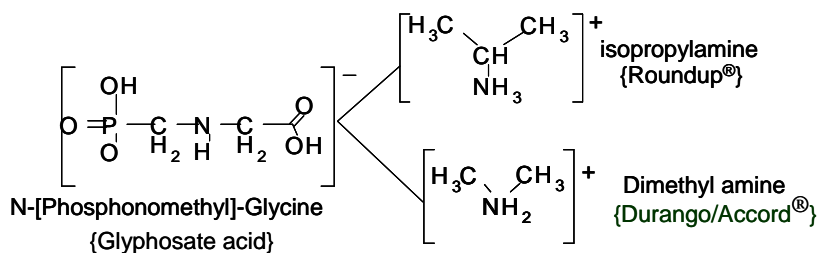
was also isolated from *Streptomyces viridichromogenes* (Thompson *et al.*, 1987). This gene encodes a metabolizing enzyme (phosphinothricin acetyltransferase) that prevents autotoxicity in the bacteria. Plants transformed with the *bar*-gene are highly resistant to glufosinate (De Block *et al.*, 1987, De Greef *et al.*, 1989). Introduction of *bar*-transformed crop plants is proceeding quickly. Gene transfer through out-crossing is an issue for glufosinate resistant canola, as the *bar* gene appears to be able to pass to closely related plants, such as wild radish in as few as four generations (Brown *et al.*, 1996).

## Inhibition of Aromatic Amino Acid Biosynthesis: Glyphosate

### Biosynthesis of Aromatic Amino Acids:

Phenylalanine, tyrosine and tryptophane are aromatic amino acids essential for protein synthesis. Biosynthesis of these amino acids (figure 2) is initiated with the condensation of a 4 carbon sugar, erythrose-4-PO<sub>4</sub>, with a 3-carbon sugar, phosphoenolpyruvate, to form a 7-carbon sugar, deoxyarabino-heptulosonate-7-PO<sub>4</sub> (DAHP), via the enzyme 3-deoxy-D-arabino-heptulosonic-7-phosphate synthase (DAHP synthase). DAHP undergoes a series of reactions, including ring closure, dehydration and reduction to produce shikimic acid. Through the action of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), shikimic acid combines with a second phosphoenolpyruvate, followed by the loss of a PO<sub>4</sub> group, to produce chorismic acid (chorismic is Greek for “fork”). This pathway has two branches following chorismate formation: one into the formation of anthranilic acid leading to the amino acid tryptophane, and the other fork leading to the biosynthesis of phenylalanine and tyrosine. Tryptophane can also be formed from serine and indolglycerol.

### Two primary glyphosate salts



### Glyphosate Mode-of-Action:

Glyphosate was identified in the late 1960's in a Monsanto discovery program that initially produced the sugar cane ripener glyphosine (which was originally identified from a Monsanto program to identifying new water softening agents). This herbicide was introduced in 1971 at the North Central Weed Science Conference. Glyphosate is a biosynthesis inhibitor of the aromatic amino acids phenylalanine and tyrosine, and these amino acids can reverse glyphosate-induced plant growth inhibition (Gresshoff, 1979). Specifically, glyphosate inhibits the enzyme 3-phospho-5-enolpyruvateshikimate (EPSP) synthase (figure 4), thus, preventing the conversion of shikimate to chorismic acid (reviewed by Duke, 1988, and Ray, 1989). Inhibition of this

biosynthetic pathway results in an unregulated accumulation of shikimate. Following glyphosate treatment, as much as 10 to 20% of the plant's total soluble carbon can be found to accumulate in shikimate. Plant death is apparently the result of the unregulated accumulation of carbon in that intermediate. As the rate of plant death is dependent on the amount of stored carbon in plant tissues, small plants may die relatively quickly (1 to 4 weeks) whereas larger shrubs or small trees may require a year or more to be fully controlled.

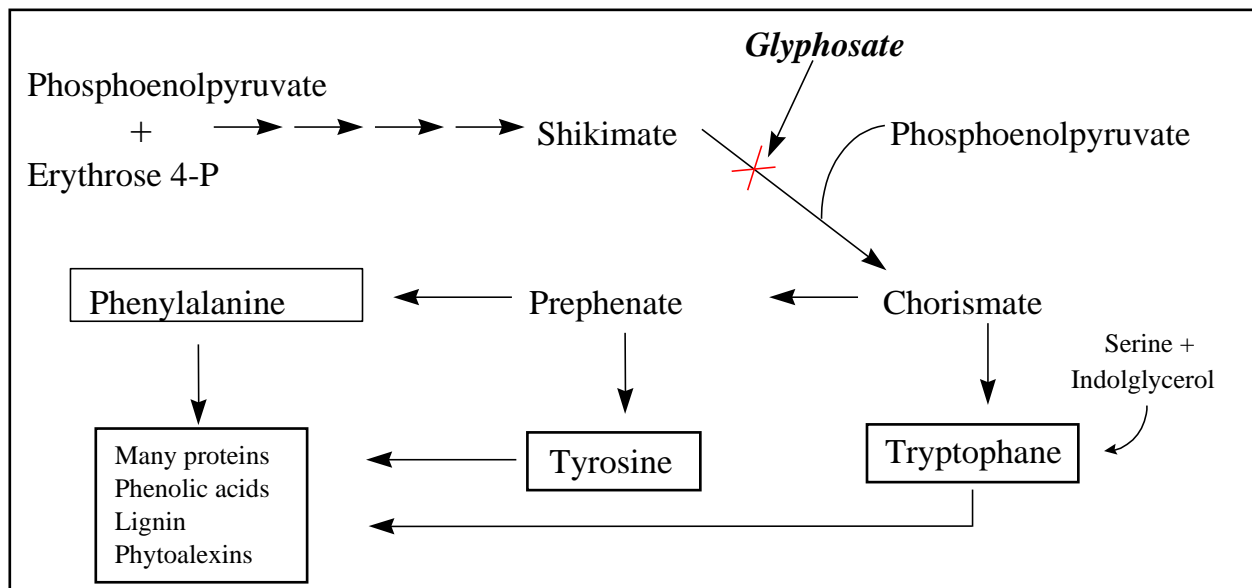


Figure 4: Aromatic amino acid biosynthesis and inhibition by glyphosate

The structure of the active site of EPSP synthase (EC-2.5.1.19) has been determined by co-crystallization of EPSP synthase from bacteria (*E. coli*) with its substrate shikimate- and glyphosate or with shikimate alone. This has allowed a determination of the structures of the enzyme-inhibitor complexes by X-ray crystallography at resolutions of 1.5 and 1.6 angstroms, respectively. Upon binding of shikimate, the two-domain enzyme closes to form an active site in the interdomain cleft. Glyphosate appears to occupy the site of the 2nd substrate, phosphoenyl pyruvate.

Two additional sites-of-action for glyphosate have been described; however, both are inhibited at far higher concentrations (mM) than required for EPSP. These sites include DAHP synthase, an earlier enzyme in the shikimate acid pathway, and the biosynthesis of 5-amino-levulinic acid (ALA), a chlorophyll precursor (reviewed by Duke, 1988). While glyphosate does inhibit chlorophyll synthesis and the whole-plant symptoms do include interveinal chlorosis, these affects appear as a result of the buildup of the shikimate (an organic acid) in the chloroplast. Accumulation of this organic acid destroys the pH balance of the plastid, causing membrane degradation and the bleaching symptomology. Young chloroplasts appear much more susceptible to glyphosate induced pH imbalance than mature plastids<sup>1</sup>. Fairly decisive evidence

<sup>1</sup> Personal communication, Dr. Douglas R. Sammons, Project Leader for Resistance Mechanisms, Monsanto Co.

that EPSP is the sole site of herbicide action can be inferred from the fact that plants genetically transformed with a glyphosate insensitive form of EPSP synthase have been shown to possess commercial levels of tolerance to the herbicide.

Activity:

Glyphosate is a non-selective broad-spectrum herbicide that is highly phloem mobile in plants. Use rates range between 0.25 and 2 lb./A. Glyphosate is not metabolized in treated plants and has no soil activity.

Glyphosate Resistant Crops

CROP	STATUS
Soybeans	Commercial
Cotton	Commercial
Corn	Commercial
Sugarbeet	Commercial
Wheat	Target~?
Alfalfa	Commercial
Canola	Commercial

Resistance to Glyphosate has been engineered into a number of crops (table 1). In these crops, glyphosate resistance is the result of plant transformation with a gene (*aroA*) coding for an insensitive form of EPSP synthase. Monsanto engaged in a significant, multi-year search to locate an EPSP synthase enzyme which both bound poorly with glyphosate, but was still biochemically efficient. Many variants of plant, yeast and bacteria EPSP synthases were characterized; a useful gene was ultimately isolated from an *Agrobacterium* bacteria. As aromatic amino acid biosynthesis occurs primarily in the plant chloroplast,

it was critical to target the gene product, EPSP synthase, to that organelle. To accomplish this, the *aroA* gene was fused to a chloroplast transit peptide sequence derived from *Arabidopsis thaliana*. The protein produced from this gene fusion product liberates bacterial EPSP synthase upon processing in the plastid. This mechanism of resistance is employed in Roundup Ready soybeans.

A second gene for glyphosate resistance has also been developed, this time coding for glyphosate metabolism via glyphosate oxidoreductase (*GOX*). The *GOX* gene was isolated from an *Achromobacter* bacteria collected from a glyphosate waste stream treatment facility. In a manner similar to *aroA*, glyphosate oxidoreductase was targeted to the chloroplast by fusing the *GOX* sequence with an *Arabidopsis thaliana* chloroplast transit peptide sequence. This gene construct has also been introduced into crop plants. Plants transformed with both the *aroA* and *GOX* gene constructs show excellent vegetative and reproductive glyphosate tolerance with little impact on yield.

Transgenic crop plants expressing glyphosate resistance have had a significant impact on US agriculture. Ninety percent or more of US soybeans and 95 percent or more of the cotton in the Southeast are glyphosate-tolerant; glyphosate-tolerant corn is catching on fast with 60% of the US acreage expressing this trait<sup>2</sup>. In the first year of introduction, canola resistant to glyphosate was planted nearly 200,000 acres, or about 20% of the total canola crop. Much of these uses

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<sup>2</sup> Service, R.F. 2007. Newsfocus: A growing threat down on the farm. Science 316; 1114-1117

were by growers who traditionally apply a soil treatment and then follow up with a post-emergence spray, with glyphosate now serving as the latter treatment. Glyphosate tolerant alfalfa was de-regulated by USDA in June, 2005, but use was halted under injunction ordered by the Ninth Circuit in September 2007 based on an appeal by non-GM alfalfa growers and environmentalist groups over fears that the GM alfalfa would cross-pollinate with conventional crops. Monsanto appealed to the Supreme Court after a divided three-judge panel on the 9th U.S. Circuit Court of Appeals upheld the ban for the second time on June 24, 2009. In its first ruling on genetically engineered crops, the Supreme Court on June 21, 2010, overturned the lower court's decision, stating that "An injunction is a drastic and extraordinary remedy, which should not be granted as a matter of course".

Monsanto had stated that the injunction was unfair to the 5,500 growers who chose to plant Roundup Ready alfalfa on some 263,000 acres (106,000 ha) of the approximately 23 million acres of alfalfa planted in the US. A very similar, if not identical situation has occurred with glyphosate-tolerance sugar beets. Sugar beets were deregulated by the Agriculture Department in 2005 following an environmental assessment and planted widely. However, in September, 2009, the Federal District Court in San Francisco ruled that the Agriculture Department should have done a more comprehensive environmental impact statement and assessed the consequences from the likely spread of the genetically engineered trait to other sugar beets or to the related crops of Swiss chard and red table beets<sup>3</sup>. However, in this case, the Federal District Court allowed plantings of glyphosate-tolerance sugar beets to continue in 2010, but warned of a potential block on the use in future seasons while an environmental review takes place. The June 21, 2010 decision for glyphosate-tolerant alfalfa may affect this case as well.

#### Glyphosate Resistant Weeds:

A significant number of examples of glyphosate resistance have been documented since 1996, including goosegrass (*Eleusine indica*) in Malaysia and rigid ryegrass (*Lolium rigidum*) in Australia and the United States (California)<sup>4</sup>. In resistant goosegrass, EPSP has been determined to be an altered enzyme with two apparent point mutations<sup>5</sup>. One is mutation (glycine → alanine) is known to inhibit glyphosate binding, but also decreases binding of EPSP to the natural substrate, PEP; the second mutation is not yet fully characterized, but may compensate for the negative fitness effects of the first. Multiple sprays at high rates still appear to provide control of the resistant goosegrass biotype. In the case of Australian rigid ryegrass, a 3X increase in the enzyme EPSP appears to be the only observed difference between resistant and wild type plants (Gruys *et al.* 1999), glyphosate-resistant annual ryegrass populations have now been confirmed at 87 sites across Australia<sup>6</sup>. The resistance trait itself may be polygenic, as a full range of tolerant and sensitive plants are found in outcross breeding events. It has been speculated that these plants contain a mutation in a chloroplast PO<sub>4</sub>-transporter that is putatively involved in glyphosate import into plastids. This hypothesis was developed from an observation of cross-

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<sup>3</sup> Judge Rejects Approval Of Biotech Sugar Beets; New York Times via NewsEdge Corporation, 24-Sep-2009

<sup>4</sup> <http://www.weedscience.com>

<sup>5</sup> Mintein Tran and Scot Baerson *et al.* 1999, Southeast Asia Weed Science Society in Bangkok.

<sup>6</sup> Dr Chris Preston, Stock and Land Journal, July 2, 2009

resistance between glyphosate-resistant ryegrass and a Zeneca AG Product's phosphonate compound that inhibits histidine biosynthesis (IUPAC, 1999). While this may be possible, the exact mechanism of glyphosate import into chloroplasts is yet unknown<sup>7</sup>. More recently, marehail with tolerance to 10 quarts/A of Roundup has been identified in the Mid-Atlantic States of Delaware, New Jersey and Maryland; waterhemp in Missouri also has at least one, but probably more, biotypes with resistance to glyphosate<sup>8</sup>. In 2003, a highly resistant population of Buckhorn Plantain (*Plantago lanceolata*) was discovered in South African vineyards with a history of poor control with glyphosate<sup>9</sup>; no mechanism has yet been postulated for this resistance. A University of Missouri weed scientist (Reid Smeda) has documented a 20-acre field in which common ragweed (*Ambrosia artemisiifolia*) has shown itself to be resistant to 10 times the rate of glyphosate that normally controls it<sup>10</sup>, this biotype is now widespread in the south. In West Tennessee, cotton producers have had to re-introduced residual herbicides into their weed control programs to combat glyphosate-resistant horseweed (*Conyza canadensis*), which now comprises between 80 percent of 90 percent of the horseweed infesting the region<sup>11</sup>. The difficulty in controlling glyphosate-resistant horseweed is exacerbated by its biology; horseweed has an extended period of germination and can emerge in all but the coldest months of the year. Glyphosate-resistant horseweed was first documented in the Mid-South in 2002 by University of Tennessee weed scientist, Bob Hayes, after it appeared in one field in west Tennessee in 2001; over the years, glyphosate-resistant horseweed has become widely distributed in Southern no-till cotton and soybeans where it has caused significant reductions in yield (Zelaya *et al.* 2007; Steckel & Gwathmey, 2009). Zelaya *et al.* (2007) has evaluated the possible occurrence of interspecies transfer of the glyphosate resistance within the genus *Conyza* and observed that hybridization and transfer of herbicide resistance can occur between *C. canadensis* and *C. ramosissima*. The researchers have determined that approximately 3% of ova were fertilized by pollen of the opposing species and produced viable seeds. The interspecific hybrids were found to have intermediate phenotype between the parents but exhibit superior resistance to glyphosate compared to the herbicide resistant *C. canadensis* parent. This fact may be responsible for the 2007 first occurrence of glyphosate resistance in the *Conyza bonariensis* that has recently been identified in California<sup>12</sup>.

There have also been confirmed reports of "Palmer pigweed" (*Amaranthus palmeri*) resistance in several states including Arkansas (Norsworthy *et al.*, 2008), Georgia<sup>13</sup> and Tennessee<sup>14</sup>. Since pigweed species are known to hybridize, glyphosate resistance in the pigweed family is of especially serious concern for if resistance in this key agronomic weed family becomes broadly entrenched across the United States, it has potential to seriously affect entire weed management and cultural systems and necessitate wide-scale changes in farming practices. In *Amaranthus*

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<sup>7</sup> Personal communication, Dr. Douglas R. Sammons, Project Leader for Resistance Mechanisms, Monsanto Co.

<sup>8</sup> Soybean Digest via NewsEdge Corporation, January 11, 2002

<sup>9</sup> WSSA International Survey of Herbicide Resistant Weeds; <http://www.weedscience.org/in.asp>

<sup>10</sup> Personal communication, Dr. Reid Smeda, University of Missouri

<sup>11</sup> Delta Farm Press, July 18, 2005

<sup>12</sup> AGROW - World Crop Protection News - <http://www.agrow.co.uk>, (A00970440) , Filed 12 September 2007

<sup>13</sup> Monsanto Imagine ([weedresistancemanagement.com/layout/press\\_releases/09-13-05.asp](http://weedresistancemanagement.com/layout/press_releases/09-13-05.asp))

<sup>14</sup> The University of Tennessee Institute of Agriculture ([agriculture.tennessee.edu/news/releases/0509-pigweed.htm](http://agriculture.tennessee.edu/news/releases/0509-pigweed.htm))

*palmeri* populations collected from Georgia, the molecular basis of resistance has been identified as EPSPS gene amplification where genomes of resistant plants contained from 5-fold to more than 160-fold more copies of the EPSPS gene than did genomes of susceptible plants (Gaines *et al.*, 2010). Interestingly, in this population, EPSPS genes were present on every chromosome; therefore, gene amplification was likely not caused by unequal chromosome crossing over.

Johnson grass resistance to glyphosate would be an agronomic problem of similar magnitude. Recently, a Johnson grass (*Sorghum halepense*) glyphosate resistant biotype has been noted in Argentina (Salta province), estimates of the effected area are 10,000 hectares and the area is increasing (De La Vega *et al.*, 2006); confirmed glyphosate resistant Johnson grass has been observed in the states of Arkansas and Mississippi; though in both cases, resistance was confined to individual farms<sup>15</sup>. Glyphosate resistance in barnyardgrass (*Echinochloa crusgalli*) is being investigated in the Lower Namoi area of Australia at the University of Adelaide where the summer fallow weed control program relied solely on glyphosate with 15 to 20 applications over a 5-year period; initial greenhouse test results have demonstrated resistance, confirmative testing is now underway<sup>16</sup>. The perennial weed, sourgrass (*Digitaria insularis*), has recently infested Paraguay's glyphosate-tolerant soybean crops and many farmers are considering a return to planting conventional seeds<sup>17</sup>. Most recently, Kansas State University scientists identified five Kochia weed populations in western Kansas with confirmed resistance to glyphosate<sup>18</sup>. Kochia, also called fireweed, is a drought-tolerant weed commonly found on land in the western United States and Canada where crops are grown and cattle are grazed.

Additionally, there have also been scattered reports of glyphosate "nonperformance" on lambsquarters (*Chenopodium album*), with the first reports appearing in South Dakota and Western Minnesota and moving east. If true, a potential mechanism of lambsquarters tolerance could be an alteration in emergence pattern in response to glyphosate-mediated selection of earlier germinating biotypes and encouragement of later germinating biotypes through reduced tillage.

A unique insect-mediated mechanism leading to reduced glyphosate performance has been reported for glyphosate in common ragweed, giant ragweed, and tall waterhemp via the disruption of vascular translocation pathways by feeding insect larva tunneling within the plant stem<sup>19</sup>. Researchers at the University of IL reported on the distribution and impact of insect tunneling on herbicidal control caused by *Lepidoptera*, *Coleoptera*, *Lixus*, and *Dectes* species. Researchers at Purdue, Michigan State and Ohio State Universities have also investigated a tunneling phenomenon in the weed species mentioned above as well as marehail. While not true resistance or tolerance, this phenomena may mimic either situation and result in misdiagnosis of the phenomena.

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<sup>15</sup> AGROW - World Crop Protection News - <http://www.agrow.co.uk>, (A00989414) Filed 17March 2008 Agrow News Update

<sup>16</sup> Glyphosate Resistance in Barnyardgrass, January 30, 2007. Cotton Tales. Vol. 6. [www.dpinsw.gov.au](http://www.dpinsw.gov.au)

<sup>17</sup> AGROW News Update, Published Online: 07-May-2008

<sup>18</sup> Associated Press via NewsEdge Corporation 1-Mar-2010

<sup>19</sup> Notes from NCWSS Herbicide Resistance Committee Meeting – Monday, December 1, 2003

Weed or volunteer populations with increased herbicide resistance are also possible due to genetic contamination of non-GM crops by glyphosate-resistant and other herbicide-resistant GM-crops. Field studies of *Brassica* pollen dispersal have indicated that most pollen falls near the release point, but some has been found up to 120 m or more from the point of release. Cultivate rape pollen is capable of fertilizing the weeds *Raphanus raphanistrum* and *Hirschfeldia incana* (Champolivier, Messean and Prunier, 2001) and the transfer of glyphosate resistance to cultivated mustards has been observed in Canada and elsewhere.

While weed population shifts to more tolerant species due to herbicide selection pressure is not true resistance, shifts to indigenous weed species with a higher natural tolerance to glyphosate and/or later emerging species has been observed following continuous use of glyphosate in crop rotation schemes limited to glyphosate-resistant crops. The grower should consider the wisdom of this type of herbicide use pattern on the long-term composition of weed populations in any agroecosystem under continuous cultivation (Miller et al., 2003).

### Inhibitors of Branched Chain Amino Acid Biosynthesis: Acetolactate Synthase Inhibitors

Acetolactate synthase (ALS) catalyzes the first committed step in branched-chain amino acid biosynthesis (figure 6, reviewed by Kishore and Shah, 1988). This enzyme facilitates the condensation of two molecules of pyruvate to form acetolactate, which is converted through a series of reaction steps into valine and leucine. ALS also catalyzes a similar reaction, producing acetoxybutyrate for the production of isoleucine, when 2-ketobutyrate and pyruvate are used as substrates. Biosynthesis of branched-chain amino acids takes place in the chloroplasts.

#### Branched Chain Amino Acid Biosynthesis Inhibition:

Five families of herbicides with remarkable activity have been discovered over the last 25 years, including the sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidinyl thiobenzoates, and sulfonylaminocarbonyltriazolinones (figure 5). These herbicides inhibit the production of branched chain amino acids by the inhibition of acetolactate synthase (ALS) (see reviews by Pesticide Science, 1990, and Stetter, 1994). Several commercial examples of these herbicide families are listed in table 2 with representative structures in figure 6.

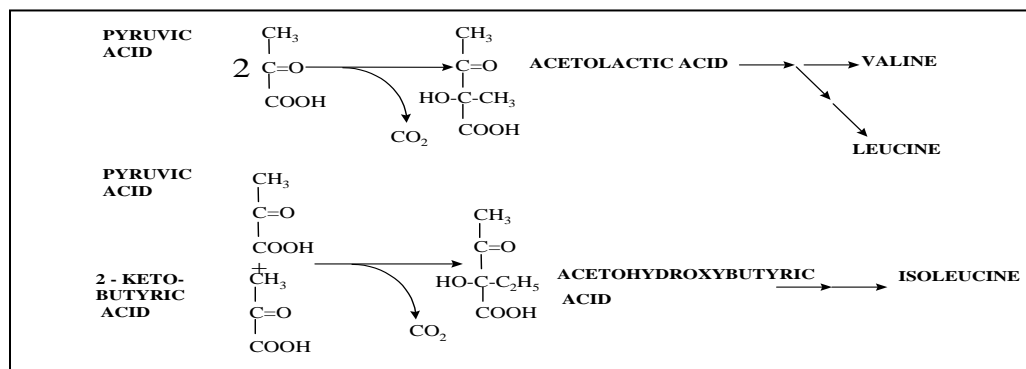
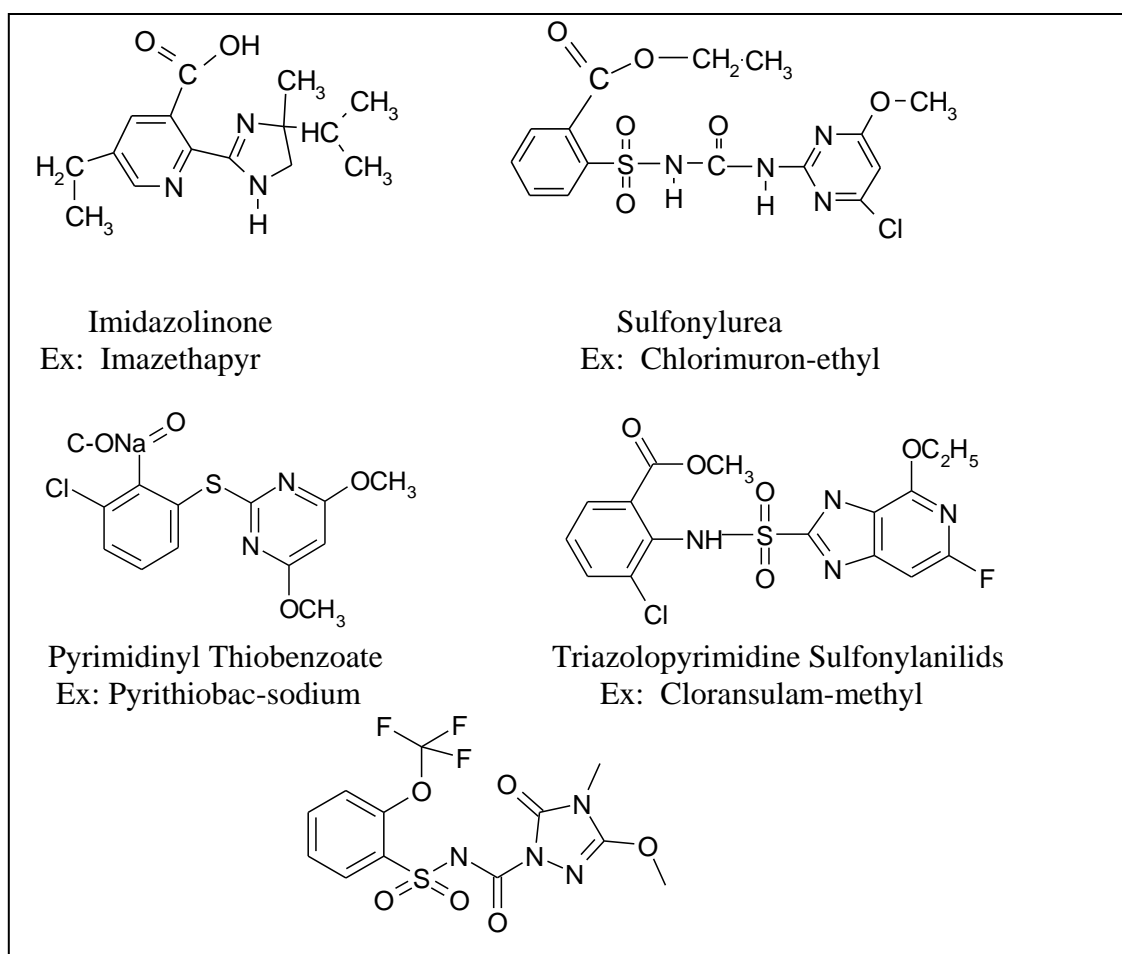


Figure 5. Reactions catalyzed by Acetolactate Synthase.

Table 2: Commercial Examples of Acetolactate synthase Inhibiting Herbicides:

Chemical Class	Trade Name	Target Crop	Common Name
Sulfonylureas	Londax <sup>®</sup> Classic <sup>®</sup> Oust <sup>®</sup>	Rice Soybean Non-Crop	Bensulfuron-methyl Chlorimuron-ethyl Sulfometuron-methyl
Imidazolinones	Pursuit <sup>®</sup> Scepter <sup>®</sup>	Soybean Soybean	Imazethapyr Imazaquin
Triazolopyrimidines	Broadstrike <sup>®</sup> FirstRate <sup>®</sup> Strongarm <sup>®</sup>	Soybean Soybean Peanuts, Soybeans	Flumetsulam Cloransulam-methyl Diclosulam
Pyrimidinyl thiobenzoates	Staple <sup>®</sup>	Cotton	Pyrithiobac-sodium
Sulfonylaminocarbonyl-triazolinones	Everest <sup>®</sup>	Wheat	Flucarbazone-sodium

Figure 6: Representative Structures of ALS-Inhibitor Chemical Families



## **Sulfonylaminocarbonyltriazolinones Ex: Flucarbazone-sodium**

### ALS-Inhibitor Mode-of-Action:

ALS-inhibiting herbicides prevent the biosynthesis of branched chain amino acids, including valine, leucine, and isoleucine through the specific inhibition of ALS (figure 5). Under laboratory conditions, ALS-inhibitor induced plant growth inhibition can be reversed by supplementing the growth medium with these amino acids. The exact mechanism-of-action resulting in plant death is unknown. Some evidence points to the buildup of one of the substrates of ALS,  $\alpha$ -ketobuterate, which may cause a general imbalance in 2-ketoacid metabolism and interfere with a variety of biosynthetic processes involved in the utilization of glucose as a carbon source (via glycolysis and the TCA cycle) (LaRossa and T.K. Van Dyk, 1987). However, more recent evidence indicates that the elevation of  $\alpha$ -ketobuterate occurs only at herbicide concentrations well above the dose required to inhibit growth in plants (Epelbaum *et al.*, 1992; Schloss, 1994). An imbalance in 2-ketoacid metabolism may be important in the inhibition of bacterial ALS and appears to be associated with intracellular acidification and the induction of a stress response (Van Dyk *et al.*, 1998). Whatever the mechanism-of-action, the suppression of branch chain amino acid biosynthesis does results in a rapid inhibition of cell division at the G1 or G2 phases of interphase in the absence of any direct affect on mitosis (reviewed by Brown, 1990). Plant growth can be inhibited within 2 hours following treatment. While cell division and growth are quickly arrested, ultimate plant death is slow. Since plant growth stops almost immediately, the competitive potential of treated weeds is not significant and the presence of affected plants in the field is of no agronomic concern. The rate of plant death is likely related to the total pool of branched chain amino acids available. Thus, small plants will succumb much more rapidly than larger species with more reserves. ALS inhibitor symptomology includes the rapid inhibition of root and shoot growth, vein reddening, chlorosis, and meristematic necrosis.

### ALS-Inhibiting Herbicide Resistant Crops:

Several herbicide resistant crops have been engineered through the mutation of the gene encoding ALS. Crops include sulfonylurea tolerant (STS) soybeans and imidazolinone resistant or tolerant (IR/IT) corn, imidazolinone tolerant (Smart<sup>®</sup>) canola, imidazolinone tolerant (Clearfield<sup>®</sup>) wheat and rice.

### ALS-Inhibiting Herbicide Resistant Weeds:

Unlike most herbicidal enzyme inhibitors, ALS-inhibiting herbicides do not bind to the catalytic domain of the target enzyme (Schloss 1990, 1994). Instead, ALS inhibitors appear to bind to an evolutionary vestige of pyruvate oxidase contained within ALS. Both pyruvate oxidase and ALS apparently share a common evolutionary origin. Since ALS inhibitors do not bind to the catalytic domain of the enzyme, some mutations in the herbicide-binding site are not lethal and have minimal selective disadvantage. This has allowed for the rapid selection of herbicide resistance by compounds with this mode-of-action.

## Amino Acid Inhibitor References

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