Lecture

Inhibition of Amino Acid Synthesis

1. General Information

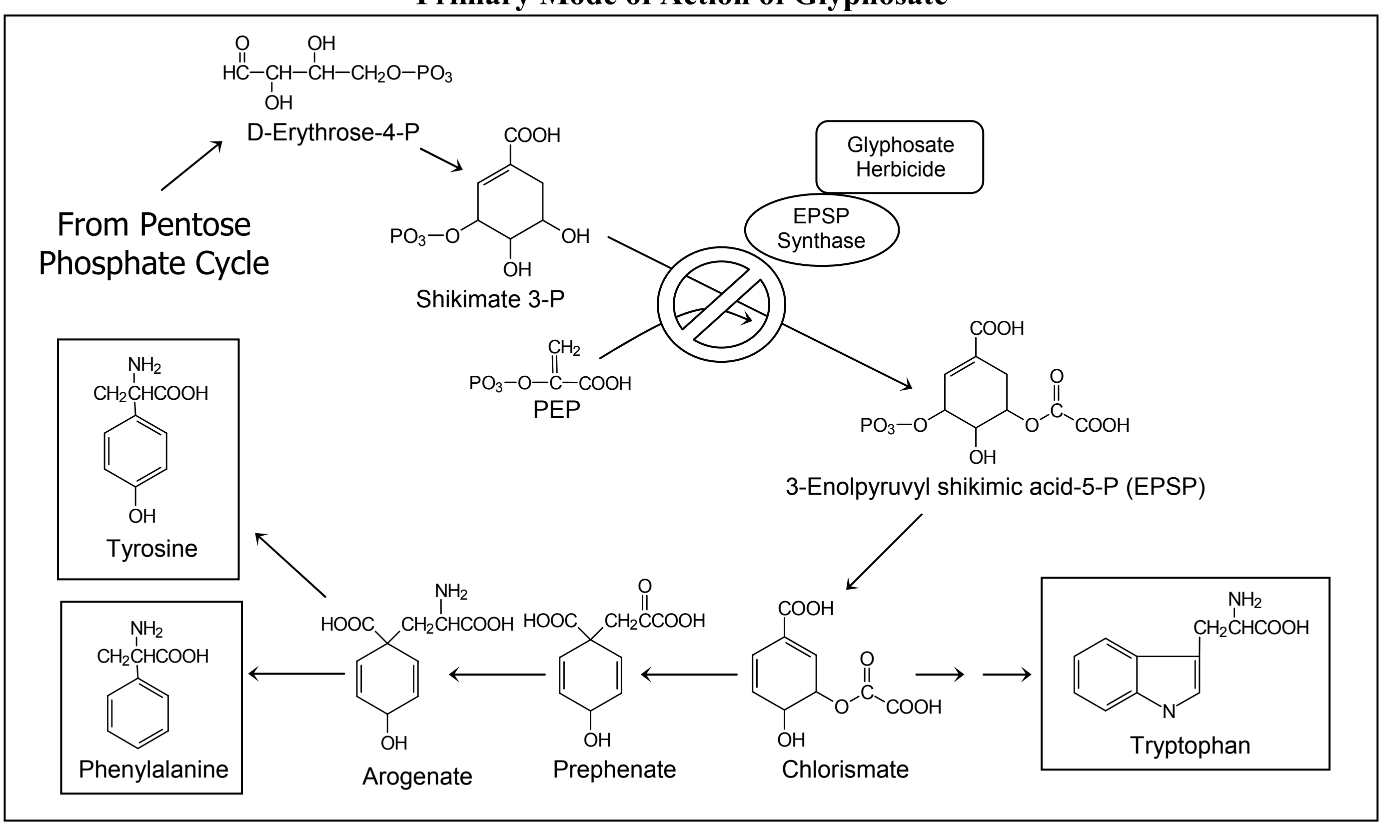
Amino acids are the building blocks of proteins. Ribosomes direct the synthesis of proteins in translation by polymerizing the amino acids in a precise order as defined by the RNA molecule.

In total, there are 20 amino acids with a variety of synthetic pathways in the plant. Herbicides that inhibit one of these metabolic pathways prevent the formation of one or more amino acids that may be required for vital plant proteins. This is accomplished by inhibiting the activity of a specific enzyme (-ase). Enzymes are catalysts that dramatically increase reaction rates of biochemical processes.

Herbicides in this group are probably some of the most important herbicides commercially, including the widely known Roundup (glyphosate) herbicide. Inhibitors of amino acid synthesis are divided into three classes – inhibitors of EPSP synthase, the ALS and AHAS inhibitors, and the glutamine synthetase inhibitors.

2. Mode of Action – Inhibition of Amino Acid Synthesis (Inhibition of EPSP Synthase)

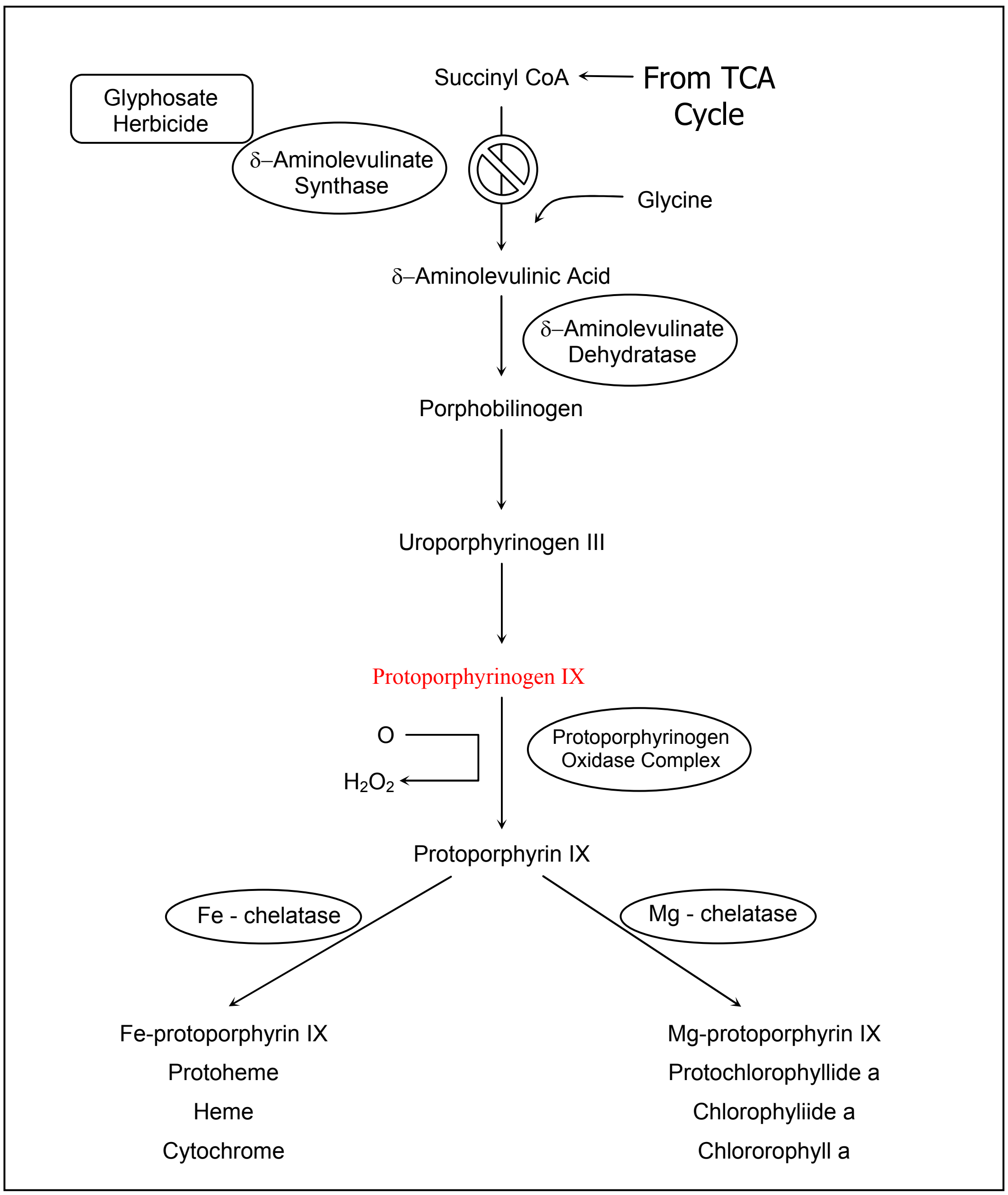
Primary Mode of Action of Glyphosate



Glyphosate kills plants by inhibiting a reaction that occurs in the shikimic acid pathway. This pathway is vital for survival of plants with the end products being several aromatic amino acids, flavenoids, lignins, anthocyanins, and coumarins. (See figure on previous page)

Glyphosate inhibits activity of 5-enolpyruvyl shikimate-3-phosphate synthase (EPSP synthase), by blocking conversion of shikimate-3-phosphate plus phosphoenolpyruvate (PEP) $\rightarrow$ enolpyruvyl shikimate phosphate (EPSP). This in turn inhibits the production of the aromatic amino acid end products tryptophan, phenylalanine, and tyrosine. Without these essential amino acids, certain proteins cannot be produced and the plant dies.

Secondary Mode of Action of Gylphosate



A secondary mode of action occurs in the aminolevulinic acid pathway, or the porphyrin synthesis pathway. In this pathway, glyphosate inhibits conversion of succinyl CoA (from the TCA cycle) to aminolevulinic acid by interfering with activity of aminolevulinate synthase. By blocking this step in the pathway, synthesis of compounds containing porphyrin ceases. This affects production of chlorophyll, cytochromes, and peroxidases, etc.

This is a secondary mode of action and in most cases, plant death occurs before results from the secondary mode of action are expressed.

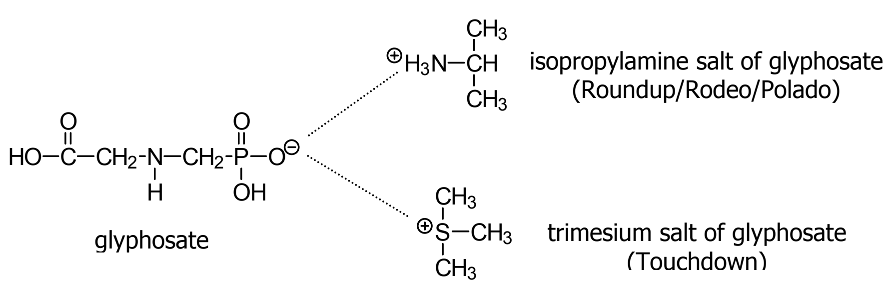
3. Site of Action – Inhibition of Amino Acid Synthesis (Inhibition of EPSP Synthase)

Glyphosate (Roundup) and trimesium glyphosate (Touchdown) act in the shikimic acid pathway, which occurs in the chloroplast. Biotypes of rigid ryegrass have become resistant to glyphosate by preventing the herbicide from reaching its site of action due to a mutated carrier protein on the chloroplast envelope.

4. Symptoms – Inhibition of Amino Acid Synthesis (Inhibition of EPSP Synthase)

- growth ceases immediately
- general chlorosis (especially in immature leaves in the growing point or apical meristem)
- necrosis within 1 to 3 weeks depending on species
- grasses are more sensitive than broadleaf weeds
- purple discoloration may occur in some species

5. Herbicide Family – Inhibition of Amino Acid Synthesis (Inhibition of EPSP Synthase)

Non-Family	
Example	
	once referred to as the aliphatics and amino acid derivatives; following absorption the salts of these herbicides ionize and the anion is the herbicidally active portion; very safe to humans with LD_{50} of > 5,000 mg/kg

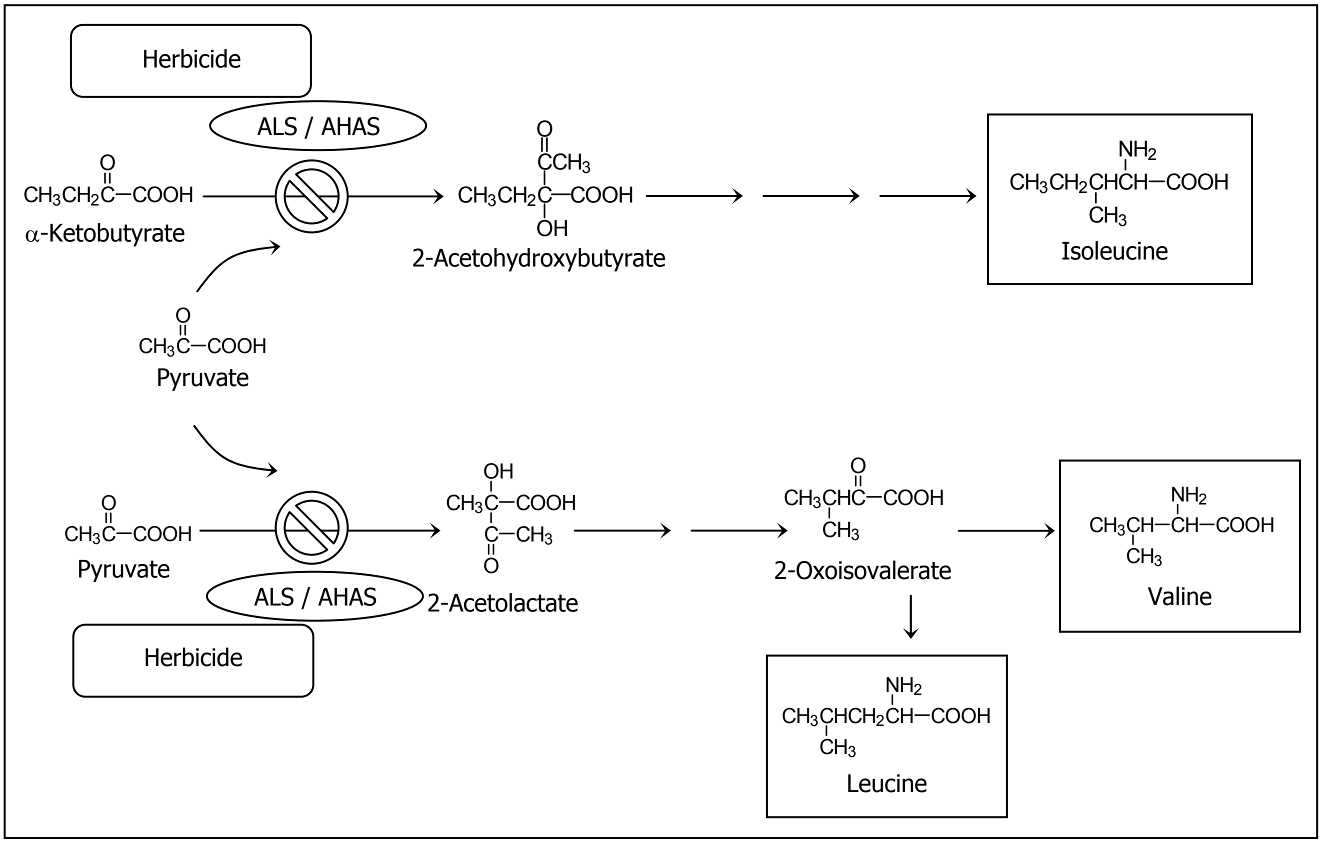
Metabolism	plant – slowly metabolized soil – microbial half-life – 47 d; all crops can be planted immediately after application due to strong adsorption by soil
Absorption & Translocation	readily absorbed by leaves and translocated in symplast with little to no movement in apoplast no soil activity
Selectivity	non-selective selectivity achieved by use of directed, shielded sprayers, or "wick bars" to avoid contact with desired plant foliage
Herbicide Use	controls annual and perennial grasses, broadleafs, and sedges used POST prior to planting or emergence of desired crop (preplant burndown), general vegetation control at industrial sites, residential, non crop areas, site preparation for Christmas trees; aquatic situations (formulation dependent); pre-harvest in cotton and wheat; recirculating sprayers, shielded sprayers, wipers; transgenic glyphosate-resistant soybean, cotton, corn, canola weed resistance issue?; GMO issue??

6. General Comments

Glyphosate, also called N-phosphono-methyl glycine, was developed in 1972 by the Monsanto Company and was first sold commercially in the late 1970's. It is a widely used, non-selective herbicide having ideal qualities of low mammalian toxicity, rapid degradation in soil, and low cost.

The introduction of glyphosate-resistant crops has revolutionized the agricultural industry, making weed control programs much simpler and in many cases less expensive.

7. Mode of Action – Inhibition of Amino Acid Synthesis (Inhibition of Acetolactate Synthase / Acetohydroxy Acid Synthase)



Herbicides with this mode of action represent four distinct chemical families, but all inhibit activity of the same enzyme. The enzyme is named differently because two agrichemical companies at the same time were developing herbicides in different chemical families with the same mode of action.

Herbicides that inhibit activity of acetolactate synthase (ALS) and acetohydroxy synthase (AHAS) do so by blocking conversion of α ketoglutarate to 2-acetohydroxybutyrate and conversion of pyruvate to 2-acetolactate. The end result is that branched chain amino acids isoleucine, leucine, and valine are not produced. Without the essential amino acid, certain proteins cannot be produced and the plant dies.

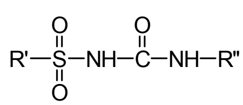
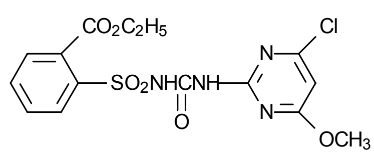
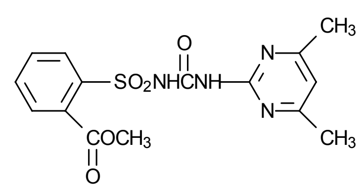
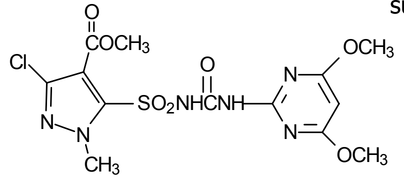
8. Site of Action - Inhibition of Amino Acid Synthesis (Inhibition of ALS / AHAS)

Herbicides in this class act on the ALS/AHAS enzyme in the branched chain amino acid biosynthetic pathway occurring in the chloroplast.

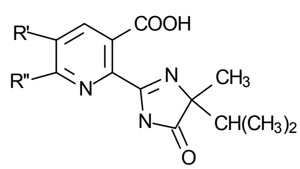
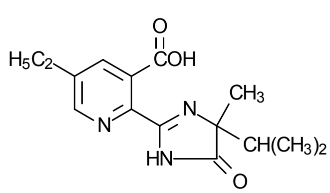
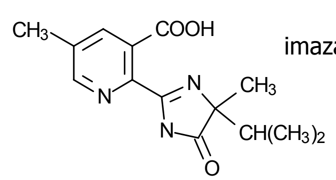
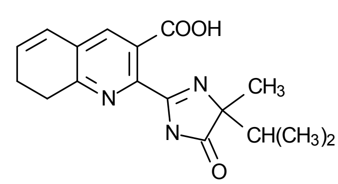
9. Symptoms - Inhibition of Amino Acid Synthesis (Inhibition of ALS / AHAS)

- growth ceases immediately if applied postemergence; shortening of internodes
- if applied postemergence, weeds can emerge and depending on species and rate will either die or remain in a stunted/non-competitive condition
- general chlorosis especially in immature leaves in the growing point / apical meristem; some grasses will turn purple
- lateral bud development is often activated resulting in abnormal branching from lower nodes
- leaves affected can be malformed
- necrosis is slow, often requiring 3-4 weeks; in some cases treated plants may persist under the canopy the entire growing season

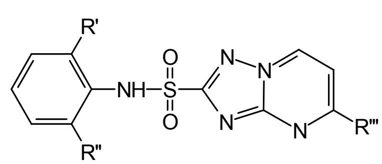
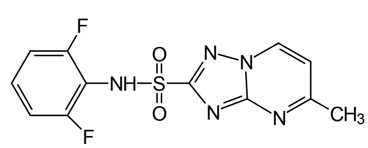
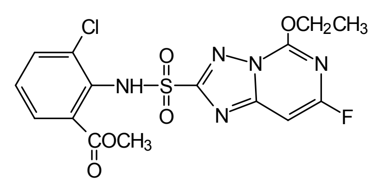
10. Herbicide Families - Inhibition of Amino Acid Synthesis (Inhibition of ALS / AHAS)

Sulfonylureas		
Base Structure		referred to as the SU's; originally discovered and developed by DuPont; urea base structure with sulfur attached to the N; many herbicides represented by several companies are in this family
Examples	 chlorimuron (Classic/Skirmish)  sulfometuron-methyl (Oust)  halosulfuron (Permit/Sempra/Manage)	
Others	bensulfuron (Londax) nicosulfuron (Accent) tribenuron (Express) prosulfuron (Peak) primisulfuron (Beacon) triflusulfuron (Up Beet) chlorsulfuron (Glean) rimsulfuron (Titus) triasulfuron (Amber) metsulfuron (Ally/Escort) thifensulfuron (Pinnacle)	

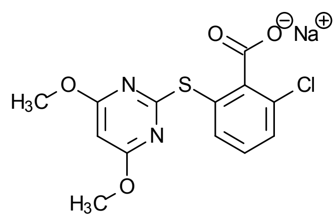
Metabolism	plant – conjugation soil – microbial (fairly slow) half-life – chlorimuron 40d; sulfometuron 20-28d; halosulfuron 4-12d; nicosulfuron 21d
Absorption & Translocation	readily absorbed by roots/shoots and foliage translocated in xylem and phloem to meristematic areas
Selectivity	selective – differential metabolism
Herbicide Use	controls annual and perennial grasses and broadleafs (dependent on specific herbicide) chlorimuron used POST in soybeans, peanuts, noncropland (first ALS herbicide) sulfometuron used PRE/POST in conifer plantings, dormant hardwood plantings, industrial sites, noncropland, airports, fence rows, lumber yards, utility rights-of-ways, unimproved turf (roadsides as a bermudagrass release); highly water soluble (problems in rice area a few years ago) halosulfuron used POST in corn, fallow ground, grain sorghum, rice, sugarcane, warm and cool season turf; very effective on sedges nicosulfuron used POST in corn (interactions with insecticides)

Imidazolinones		
Base Structure		referred to as the Imi's: discovered and developed by American Cyanamid (currently BASF); base structure contains a 5 member imidazole ring with methyl and isopropyl radicals bonded to the 4-position and an O double bonded to the 5-position; herbicides differ in the substitution at the 2-position of the ring
Examples	imazethapyr (Pursuit) imazapyr (Arsenal) imazaquin (Scepter/Image)	
Others	imazamethabenz (Assert) imazamox (Raptor) imazapic (Cadre)	

Metabolism	plant – hydroxylation, conjugation, photolysis soil – microbial half-life – imazethapyr 60-90 d; imazaquin 60 d; imazapyr 25-142 d
Absorption & Translocation	readily absorbed by roots/shoots and foliage translocated in xylem and phloem to meristematic areas
Selectivity	selective – differential metabolism
Herbicide Use	controls annual and perennial grasses and broadleafs (herbicide dependent) imazethapyr used POST in soybeans; Imi-resistant (Clearfield) corn and rice (in development); in Clearfield corn, Lightning herbicide (imazethapyr + imazapyr) is used; edible legumes, peanuts, alfalfa; excellent on johnsongrass and velvetleaf imazaquin used PRE/POST in soybeans; established turf (Image); carryover to rotational crops can be a problem; cocklebur resistance imazapyr used PRE/POST in noncropland areas such as fence rows, farm building sites, pipeline and highway right-of-ways, utility and pumping stations, roadsides, non irrigation ditchbanks, hardwood control in pines (pine release); more active than other imi's

Triazolopyrimidines		
Base Structure		triazole refers to the 5 member ring with 3-N; a pyrimidine has 2-N's in the 6 member ring structure; unlike herbicides in the other families, these herbicides control broadleafs and provide almost no grass control
Examples	 flumetsulam (Python)	 cloransulam (FirstRate)
Metabolism	plant – conjugation soil – microbial half-life – flumetsulam 1-3 months; cloransulam 8-10 d	
Absorption & Translocation	absorption by roots and by emerging shoots readily translocated from roots upward	

Selectivity	selective – differential metabolism
Herbicide Use	controls broadleafs flumetsulam used preplant, PPI, or PRE in corn and soybeans; strength in South is prickly sida (Teaweed) control; premixes include Frontrow (flumetsulam + cloransulam) POST in soybeans; POST in corn with premix of Hornet (flumetsulam +clopyralid) cloransulam used PRE/POST in soybeans; strength is prickly sida and sicklepod control

Pyrimidinylthio-benzoates		
Example	 pyrithiobac (Staple)	pyrimidine refers to a 6 member ring with 2-N's; thio to the S attached; benzoate refers to a benzene ring with carboxylic acid (-ate) group attached (hence a benzoate); this herbicide was purchased from Kumai Company (Japan) and is marketed by DuPont; this family is sometimes referred to as the benzoates
Metabolism	plant – metabolism not reported soil – microbial (major), photodegradation half-life – not available	
Absorption & Translocation	absorbed by roots and foliage translocated in phloem	
Selectivity	selective – differential metabolism	
Herbicide Use	controls broadleafs (suppresses yellow nutsedge); some grass control pyrithiobac used PRE and POST in cotton	

11. General Comments - Inhibition of Amino Acid Synthesis (Inhibition of ALS / AHAS)

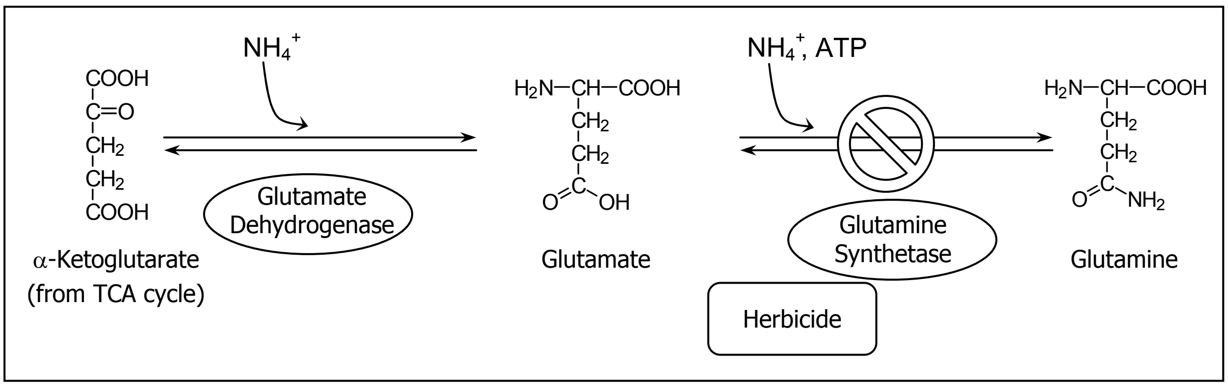
Introduced in the 1980's, the sulfonylureas (SU's) and imidazolinones (imi's) were the first ALS/AHAS inhibitors sold commercially. The SU's were developed by the Dupont company and at the same time, the imi's were being developed by American Cyanamid, now BASF.

Dupont describes the enzyme their herbicide family inhibits as the acetolactate synthase enzyme, while American Cyanamid claims their chemistry inhibits the acetohydroxy acid synthase enzyme. In truth, both chemistries inhibit the same enzyme, the companies just choose to call it by a different name.

Most of the ALS/AHAS inhibitors are used for selective postemergence control, however, some are used as selective preemergence herbicides as well. Most all of these herbicides suppress nutsedges and depending on herbicide, control some grasses.

These herbicides are used at very low use rates (oz/A) and may cause persistence problems to successive crops. In general, the SU's are used at lower rates than the imi's.

12. Mode of Action – Inhibition of Amino Acid Synthesis (Inhibition of Glutamine Synthetase)



Glufosinate kills plants by inhibiting the conversion of glutamate + $NH_4^+ \rightarrow$ glutamine. The production of the amino acid glutamine is essential to plant growth. Specifically the herbicide inhibits the activity of the enzyme glutamine synthetase preventing the normal reaction from occurring.

Since ammonium nitrogen (NH_4^+) cannot be assimilated, it builds up in the cell and interacts with membranes, which destroys cells. Also, in conditions conducive to photorespiration (high O_2, low CO_2), photosynthesis is inhibited. The conversion of α-ketoglutarate to glutamate, a necessary reaction in the photorespiratory pathway, is drastically thrown out of balance and hence, photosynthesis shuts down.

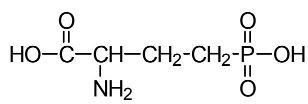
13. Site of Action - Inhibition of Amino Acid Synthesis (Inhibition of Glutamine Synthetase)

The ammonia metabolism pathways occur in the chloroplast and in the cytoplasm of green tissues. The pathway also occurs to a lesser extent in the cytoplasm of root tissues.

14. Symptoms - Inhibition of Amino Acid Synthesis (Inhibition of Glutamine Synthetase)

- chlorosis and wilting within 3 to 5 days following postemergence application
- necrosis in 1 to 2 weeks
- symptom development increases in bright sunlight, high humidity, and moist soil

15. Herbicide Family - Inhibition of Amino Acid Synthesis (Inhibition of Glutamine Synthetase)

Non-family		
Example	 glufosinate (Liberty/Finale/Ignite)	once referred to as the aliphatics and amino acid derivatives; this herbicide is formulated as an ammonium salt ($R\text{-}CH_2\text{-}P\text{-}O^{-}\,{}^{+}NH_4$); glufosinate has been in development longer than any other herbicide that I am aware of; no soil activity
Metabolism	plant – metabolism not reported soil – microbial (rapid degradation) half-life – 7 d	
Absorption & Translocation	little to no absorption by roots because of rapid microbial breakdown absorbed readily by leaves, but movement in xylem and phloem is limited	
Selectivity	nonselective	
Herbicide Use	controls annual and perennial grasses and broadleaf weeds used POST in noncrop areas, directed spray in field-grown and container nursery stock; transgenic crops i.e. Liberty Link technology in soybeans, corn, rice (in development); chlorosis and necrosis occur much more rapidly than with other herbicides that inhibit amino acid synthesis; death due to ammonia toxicity rather than amino acid depletion	

16. General Comments - Inhibition of Amino Acid Synthesis (Inhibition of Glutamine Synthetase)

Glufosinate, also known as phosphinothricin, was first reported as a herbicide in 1981, but it was not released in the U.S. until 1994. The herbicide, as well as some closely related compounds, were first found to be produced by certain species of Streptomyces bacteria.

During the transgenic explosion of the 1990's, studies reverted back to these species of bacteria to obtain the BAR and PAT genes, both of which impart glufosinate resistance in transformed plants. These genes not only have proven useful for transgenic crops, but have also been useful as "stacked gene" selective markers for inserting other, non-related genes into plants.

17. References

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