Understanding Herbicides: What They are and How They Work

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Herbicides are chemical compounds which damage or kill plants. In most environments, herbicides can be used to provide selective control of weeds without injuring the crop or other desirable vegetation. In other areas, such as roadsides or industrial sites, non-selective herbicides or a combination of herbicides can provide total vegetation control.

The discovery of new herbicides is an expensive and time-consuming process. It requires the synthesis of thousands of chemicals in industry laboratories and screening of these chemicals on an array of monocotyledonous and dicotyledonous species, including crops and weeds. Those compounds which show potential for an intended use are further tested in both the greenhouse and the field. At this time a series of toxicological and environmental fate tests must be performed prior to submission for registration. Once these battery of tests and trials have been completed, a registration package is submitted to the federal Environmental Protection Agency (EPA). If a compound is to be registered in California, a separate registration package must be submitted to the California Environmental Protection Agency, Department of Pesticide Regulation (DPR). Once registered, herbicide are classified as general-use or restricted-use pesticides. The use of restricted-use pesticides requires that they be applied by a certified applicator.

The registration of herbicides, especially in California, is constantly changing as laws, rules, and regulations are modified. In this chapter, every attempt was made to provide the most up-to-date registration information. A list of herbicide registered in the United States and

California are provided in Table 1. Due to regulatory changes and new registrations, it is expected that some compounds will loss their state and/or federal registration, while other compounds will be added to this list. Thus, it is important that the user refer to the DPR Internet site (www.cdpr.ca.gov/docs/database/database.htm) or check more recent information (e.g., www.ipm.ucdavis.edu or wric.ucdavis.edu) on current herbicide registrations.

HERBICIDE CLASSIFICATION SYSTEMS

Herbicides have been classified in several different ways, including usage, translocation patterns, method of application, selectivity, time of application, plant symptomology, toxicology, mode of action, and chemical structure. The usefulness of each classification methods depends upon the desired objective of the user. For example, an applicator may find herbicides organized by usage, or time and method of application to be more useful than systems classifying herbicides by translocation patterns, chemical structure or mode of action. Conversely, a researcher or chemist would be more concerned with classification methods based on mode of action or chemical structure. Table 2 provides a list of several characteristics of herbicide registered for use in California at the time of publication. This includes information on chemical, trade and herbicide class, as well as general information on herbicide uptake, translocation, method of application, and selectivity. Additional information can be obtained by reviewing the Herbicide Handbook (1994), other weed science text books, individual plant labels, or the DPR Internet site.

Usage

For the grower or applicator, usage provides one of the most important way of classifying herbicides. There are herbicides registered for application in non-crop areas, including aquatics, rangeland, pastures, wildlands, rights-of-way, recreational areas, utility sites and forests, and herbicides specific for turf, ornamentals, and individual agronomic, vegetable, and horticultural crops. For information on what herbicides are registered for crop and non-crop areas, consult the DPR Internet site.

Contact or Systemic

A contact herbicide moves very little in plants and kills only plant parts in close proximity to the point of chemical contact. Although it is commonly stated that contact herbicides do not translocate, in some cases they can be quite mobile. For example, paraquat can readily translocate in plant tissues, but is classified as a contact herbicide because of its rapid action when applied to the foliage. For contact herbicides to be effective, adequate distribution of the herbicide over the foliage is essential. Selectivity may depend upon the arrangement and angle of leaves, differential wetting, presence of a surfactant, the location of the growing points, or spray placement. Contact herbicides are most useful for control of annuals, or perennials in the seedling stage.

Systemic herbicides kill plants by translocating the active molecule to sites not directly in contact with the spray solution. When applied to the foliage, for example, the herbicide is translocated from the mature leaves to areas of the plant utilizing higher amounts of energy. This includes the root and shoot growing points, underground structures (i.e., rhizomes, tubers, bulbs) and reproductive structures. For this reason, herbicides of this type are effective on perennial plants, as well as annuals.

Apoplastic Translocation

Systemic herbicides are primarily translocated in xylem (apoplast) or the phloem (symplast). Table 2 lists the primary pathway of movement for herbicides registered in California. The apoplast is defined as the non-living portions of the plant. This includes the cell walls and the xylem. The apoplasm is the primary pathway that water moves from the soil to the foliage. The driving force for this upward movement is the removal of water from the leaves by transpiration. Transpiration of water from the leaves acts as a wick, bringing more water up from the roots.

When plants are under drought stress the stomata close to reduce water loss through transpiration. This reduces the movement of water from the roots to the leaves and protects the plant against desiccation. The consequence of this is that carbon dioxide cannot enter the plant. Hence, photosynthesis is slowed and growth is reduced. Although a herbicide may be considered apoplastically mobile, it must at one time enter the symplasm to exert its phytotoxic effect.

Symplastic Translocation

The symplasm consists of the living tissues of the plant. This encompasses the network of connecting cytoplasms throughout the plant, including the phloem. Unlike xylem cells, the phloem is considered living tissue because each cell contains a nucleus. It is primarily within the plant cell symplasm that sugars move from photosynthetically active tissues (source areas), principally the leaves, to growing tissues including the apical meristems, expanding young leaves, rapidly elongating stems, developing fruits and seeds, and root tips. Sugars can also accumulate in storage tissues, particularly underground root crowns, taproots, tubers, rhizomes and bulbs. All of these

storage and growing areas are referred to as sinks. Symplastically mobile herbicide move along the same pathway as sugars. The driving force for movement of sugars in the symplasm is the sucrose pressure gradient from areas of high concentration at the source to lower concentrations at the sinks.

The movement of a herbicide a will depend on where it is applied (soil or foliage) and how it is translocated in a plant. If an apoplastically mobile herbicide is soil applied the herbicide will accumulate in the leaves. Most of the herbicide will appear in the older leaves which are actively transpiring, although there will also be a certain amount of transpiration to the young leaves. If the same herbicide was applied to the foliage it would remain in the leaves and would not move to the roots under normal conditions. Herbicides which are photosynthetic inhibitors are usually apoplastically mobile, as are many soil applied herbicides.

A symplastically mobile herbicide applied to the foliage are distributed to the growing points or underground perennial structures. The same herbicide applied to the soil would probably not translocate much. Most symplastically mobile herbicides are applied to the foliage. Nearly every herbicide moves both symplastically and apoplastically to some degree.

When a perennial plant with underground reproductive organs is treated with a postemergence symplastically mobile herbicide, translocation of sugars and the herbicide to belowground parts is most rapid when large amounts of food reserves are moved towards the roots. This usually occurs after full leaf development. Postemergence application of a symplastically mobile herbicide to perennials when they were just beginning to emerge will not result in translocation of the herbicide to the vegetative reproductive structure. Thus, timing of foliar herbicide application is important for the control of perennial weeds.

Maximum control of the underground parts of perennials also depends on the maintenance of live phloem cells. Increasing the herbicide concentration with the intention of getting better

results would likely kill the phloem cells and stop translocation into the underground parts. The above ground portions of the plants would quickly die, but plants would recover as little herbicide would accumulate in the vegetative reproductive structures.

Method of Application

Herbicides can be classified by the way they are used, such as soil-applied, foliar-applied, soil or stem injection, basal bark, cut stump, and treatment directly into water (aquatic weeds or chemigation). The method of herbicide application will depend upon several factors, including its avenue of uptake into the plant, pathway of translocation, and site of action.

To be effective, herbicides must enter the plant. This can occur when roots, seeds, and emerging cotyledons, coleoptiles, or hypocotyls are exposed to soil-applied herbicide or when buds, stems, and leaves are treated with a foliar herbicide. The primary site of uptake for herbicides registered for use in California is listed in Table 2.

Root Absorption

Roots are the most important site of soil-applied herbicide uptake. Since the function of roots is nutrient and water uptake, herbicide entry is not as limited in roots as penetration into leaves. Herbicides in soil solution co-migrate with water taken into the root tip or root hairs of the plant. As a herbicide moves through the root cell wall towards the vascular cylinder it will encounter the Casparian strip. This is a watertight waxy barrier (impregnated with suberin) in the cell walls of the endodermis. For herbicides to bypass the Casparian strip they must be transported across the cell membrane (plasma membrane) on the outside of the barrier (endodermis) into the cytoplasm. At this point the herbicide can either remain in the intercellular

region or move back out of the cell once it have passed the Casparian strip. Xylem (apoplastically) mobile herbicides appear to move very rapidly across the plasmalemma. Thus, the Casparian strip does not serve as an important barrier to absorption or translocation. The reason these herbicides move predominantly in the apoplast is that they are also capable of readily moving back out to the cell wall and xylem. Since the transpiration stream is considerably faster that solute movement in the phloem, these herbicides are washed away in the xylem and, consequently, be predominantly apoplastically mobile. The majority of soil applied herbicides translocate via the apoplasmic pathway, thus they ultimately accumulate in the shoot tissues, e.g., triazine and ureas herbicides.

Uptake by Seeds and Emerging Hypocotyls, Coleoptiles, and Cotyledons

Hypocotyls are typically associated with broadleaf species. It is the portion of the seedling stem below the cotyledon(s). The coleoptile is a leaf-like sheath that protects the shoot tip and leaves of a grass seedling as it emerges from the soil. These structures can be important avenues for the absorption of many low solubility, lipophilic soil-applied herbicides that translocate to only a limited degree in plants (e.g., EPTC, trifluralin). These young tissues lack the well developed waxy cuticles, typical of more mature leaves, as well as a Casparian strip. This makes herbicide penetration into these tissues relatively easy.

Herbicides can also be adsorbed or absorbed by seeds prior to or during seed germination. These absorbed herbicides can later be taken up by the seedling. This is the case with the sodium salt of 2,4-D.

Stem Absorption

The direct application of herbicides by stem injection, basal bark treatment, cut stump application is generally used for control of woody plants. In these cases, there are three types of herbicide treatments. These treatments provide direct access to the vascular tissues. All herbicides applied by this method translocate via the symplasm and accumulate in the below and above ground growing points (e.g., glyphosate, triclopyr, imazapyr).

Foliar Absorption

Herbicides applied to the foliage can move into the plant through 1) cracks in the leaf surface, 2) open stomata, or the 3) leaf cuticle. The cuticle is the most important means of direct penetration of herbicides into the leaf. Even contact herbicides must be able to penetrate into the cell through the cuticle in order to be active. The cuticle is a thin waxy layer on the outer wall of epidermal cells. Its primary function is to protect leaf surfaces from water and gas loss. In addition to preventing loss of water, it also acts as a significant barrier to the penetration of water soluble herbicides.

The cuticle is composed of three distinct substances. Each of these layers is to some degree, intermeshed with its adjacent layer. The outer layer is the cuticular wax. It is very lipophilic and non-polar. Cuticular waxes are composed of two types, epicuticular and embedded. The epicuticular wax is the most significant barrier to the penetration of water soluble herbicides. However, lipophilic oil soluble or ester herbicide formulation easily penetrate this waxy layer by simple diffusion. The cutin comprises the bulk of the cuticle. It is more hydrophilic and polar than the cuticular wax. Cutin will hydrate in the presence of water and is not considered a major barrier to lipophilic or particularly hydrophilic herbicide movement. The innermost layer is the pectin.

Pectin strands are located at the cutin/cell wall interface or dispersed within the cutin layer. They are very hydrophilic and, like cutin, provide pathways for water soluble herbicides when hydrated.

Non-polar herbicides, such as ester formulations can easily penetrate the cuticular waxes and move through the embedded waxes. Most herbicides are polar and are applied in an aqueous form, e.g. sethoxydim, bentazon, and amine formulations of growth regulator herbicides. These are less likely to penetrate the epicuticular waxes. In these cases a surfactant is added to the formulation to increase uptake.

Once a herbicide has penetrated the cuticle it encounters the cell wall, composed primarily of cellulose. Both polar and non-polar molecules can easily move through the cellulose, as it is very porous. The final barrier for entry of the herbicides into the cell is the cell membrane. Neutral herbicides (no charge) readily penetrate the cell membrane by simple diffusion. Other herbicides may carry a negative or positive charge. These compounds move across plant membranes at a much slower rate. The rate of transport may be limited by a protein transport carrier. Evidence suggests that the uptake of 2,4-D, glyphosate, glufosinate, and paraquat into cells is facilitated by specific proteins embedded within the cell membrane. Once these herbicides have entered the cell they do not move back into the apoplast to any degree. Therefore, they are likely to be translocated within the symplastic pathway.

Many foliar-applied herbicides diffuse across cell membranes as neutral molecules, but quickly dissociate to form a negatively charged compound. These herbicides are called weak acids. Negatively charged herbicides are not freely permeable through the plant cell membrane. Thus, these compounds are trapped in the cell. Weak acid herbicides are always translocated primarily via the symplastic pathway.

Any environmental condition which directly effects the cuticle will have some effect on herbicide absorption. For the most part, maximum weed control will occur in a foliar-treated herbicide applied under warm, humid conditions with adequate soil moisture. In contrast, minimum control generally occurs when plants are water-stressed at cool temperatures with low humidity. Environmental factors often account for much of the inconsistency in the performance of foliar-applied herbicides.

Selectivity

Selective herbicides are more toxic to some plant species than others at the normal rate of application. Although many herbicides are selective to broad taxonomic groups, such as grasses (e.g., 2,4-D, dicamba, triclopyr) or broadleaf species (e.g., bromoxynil, bentazon, sethoxydim), others control a selected group of both grass and broadleaf species (e.g., alachlor, atrazine, napropamide). Contact herbicides are primarily selective on annual species (e.g., paraquat, pelargonic acid). A few herbicides are considered non-selective and control nearly all species (e.g., glyphosate, tebuthiuron). Broad categories of herbicide selectivity are listed in Table 2.

Most herbicides can kill crops or desirable vegetation under a particular set of conditions. What determines selectivity is the total amount of herbicide that reaches a sensitive metabolic site. There are a number of complex interactions between *plants*, their *environment*, and the *herbicide* that can influence selectivity of a plant to a particular herbicide. It is important to recognize the selectivity is generally rate-dependent. Over-application of a herbicide will often result in loss of selective control.

Time of Application

The time of herbicide application may largely determine its usefulness in crops. Timing of application will depend upon several factors including the crop sensitivity, weed composition and growth stage, chemical characteristics or effectiveness of the herbicide, and environmental conditions, particularly rainfall. For herbaceous weed control in non-crop systems, herbicides are applied preemergence or postemergence to the weed(s). Woody plants are controlled by postemergence applications to the foliage or bark, or by direct stem or root injections. In crops, herbicides are applied either preplant, preemergence, or postemergence to the crop and/or the weeds. A list of application timings are included in Table 2.

- Preplant (PPI) treatment is any application made before the crop is planted, usually in the final stages of seedbed preparation. If the compound is incorporated into the soil it is called preplant incorporated (PPI). Herbicides with very low solubility, sensitivity to photodecomposition, and/or high volatility (vapor pressure) require incorporation (e.g., trifluralin, EPTC, metham).
- Preemergence (PRE) treatment is the application of a chemical before or at crop seeding, after planting but prior to crop emergence, at time of transplanting, as a lay-by treatment in established annual crops, or to established horticultural crops, such as trees and vines. These compounds are not typically susceptible to volatilization or photodegradation, but do require incorporation by irrigation or rainfall.
- Postemergence (POST or POE) treatment is the application of a chemical to the foliage of weeds or the crop after emergence. Postemergence applications can be made at the early seedling stage (early POST), or to more established plants (late POST).

Plant Symptomology

Herbicide symptomology can be classified into growth suppression or deformation, chlorosis and necrosis, and growth regulator-type injury. The type of injury induced by herbicides is usually a function of its mode of action. This will be discussed in more detail in a later section. Additional information regarding injury symptoms can be found in the Plant Diagnosis section of Chapter 8 on Chemical Control Methods.

Toxicology

Several toxicological tests are conducted on each herbicide. From these tests, a variety of hazard indicators values are derived. The most commonly used of these include an Oral LD_{50}, Inhalation LC_{50}, and Dermal LD_{50}. A LD_{50} or LC_{50} value is the lethal dose or concentration of a herbicide which will kill 50% of the test animals based on mg of chemical per kg of body weight. These values and other are used to classify herbicides and specific formulations into toxicity categories. The signal words “DANGER”, “WARNING”, or “CAUTION” will appear on labels of herbicides, depending upon their toxicity profile. Changing laws, rules, and regulations, as well as new trends in herbicide development over the past couple of decades have led to a higher percentage of registered herbicides with a CAUTION Signal Word (least toxic) compared to herbicides available in 1970 or earlier (Table 3).

Chemistry

Like plants, insects and animals, chemicals share various properties that enable them to be classified into specific families or classes. In most cases, herbicides in the same chemical family will inhibit plant growth by similar mechanisms. This is not always the case, however. There are examples of insecticides, fungicides, and other pesticides that belong to the same

family as herbicides. This is because they share similar important chemical characteristics. Some herbicides are unique and are, therefore, considered to be either unclassified or in their own family. Occasionally a quality of a family may make it easy to visually recognize. The dinitroanilines are always yellow or orange in color, both as a solid and as a liquid. A list of chemical families, base structures, and the herbicides associated with each are included in Table 4.

MODE OF ACTION

There are a number of biochemical responses which may be affected by herbicides. A given herbicide may initially interfere with a single biochemical reaction or may be relatively non-specific and interfere with several reactions simultaneously. Most of the biochemical reactions that may be altered by herbicides involve one or more of seven plant metabolic processes; photosynthesis, respiration, and carbohydrate, lipid, protein, nucleic acid, amino acid, or cell wall metabolism. By disrupting any of these biochemical reactions, herbicides may injure or kill the plant. A few herbicides may have a direct effect on membranes without inhibiting specific biochemical processes. Although the activity of most herbicides is well understood, little is known of the mode of action of a number of commonly used herbicides.

A partial list of herbicides exhibiting growth regulator activity, inhibition of photosynthesis, pigment, lipid, cell wall, and amino acid synthesis, cell division, energy production, membrane destruction, and general cellular death is presented in Table 5.

Growth Regulators

Growth regulator herbicides are compounds which mimic naturally occurring auxins. These can be divided into several groups; indole acids, phenoxy carboxylic acids, benzoic acids,

and picolinic acid derivatives. The indole acids include the naturally occurring growth regulator indole acetic acid (IAA). No compounds within this group commercially available as herbicides. The latter three groups contain many well known herbicides including the phenoxy carboxylic acids 2,4-D, 2,4-DB, dichlorprop, MCPA, and mecoprop, the benzoic acid dicamba, and the picolinic acids (also known as pyradines) clopyralid and triclopyr. These compounds are often called auxinic herbicides. Quinclorac has also been shown to have growth regulator activity on broadleaf species, although it is not typically considered to be a auxinic herbicide.

Auxins regulate plant cell growth and differentiation. Their specific mechanism of action is still not well understood. However, the initial response of plants to auxin treatment can be categorized into two phases. First, there is a fast response (within minutes), characterized by rapid acidification and loosening of the cell wall. The second phase of the response occurs 30-45 min after treatment, and involves the synthesis of nucleic acids.

The biochemical and metabolic changes in plants reported to be induced by auxinic herbicides are numerous. Among these include, leaf chlorosis, altered stomatal function, stem tissue proliferation, root initiation in stem tissue, disintegration of root tissues, and abnormal apical growth. Many of these are secondary effects. The primary effect of low levels of growth regulator herbicides on nucleic acid synthesis appears to be a stimulation of RNA polymerase followed by a stimulation in RNA and protein synthesis. However, in meristematic tissues, high levels of auxins inhibit RNA synthesis and growth. In contrast, high auxin levels stimulate RNA and protein synthesis is mature tissues. The abnormal stimulation of cell division by synthetic auxin treatment, in conjunction with the rapid cell wall loosening response, leads to uncontrolled growth and eventual collapse of the vascular tissues.

A characteristic twisting symptom known as epinasty occurs following treatment with all of the auxin-like herbicides. This response is the result of a auxin-induced stimulation in ethylene production. This symptom, in itself, is probably not responsible for the phytotoxic activity of these herbicides.

Photosynthetic Inhibitors

Photosynthesis is a complicated process in which CO_2 and water are converted to oxygen and organic compounds, particularly sucrose. These events occur primarily in the chloroplasts of leaf cells. What drives this reaction is the conversion of light energy to a more usable form of chemical energy, as ATP and NADPH. Thus, light is required for both photosynthesis and the activity of all photosynthesis inhibiting herbicides. Photosynthetic inhibitors can be divided into two groups, those that inhibit the transfer of electrons in photosystem II and compounds which accept electrons from photosystem I.

Electron transport inhibitors

In the light reaction of photosynthesis, chlorophyll and other pigments that absorb light photons are momentarily raised to a higher energy state. The electrons from these energized molecules pass along a chain of membrane-bound carriers to NADP+. Several classes of herbicides inhibit this electron transport process in photosystem II. These include some amides, nitriles, and pyridazinone herbicides, including propanil, bromoxynil, pyrazon, and pyridate, and the benzothiadiazole, phenyl-carbamate, triazine, triazinone, uracil, and urea herbicides. These compounds bind to a protein (D-1) at the binding niche for a protein-bound plastoquinone molecule

(Q). When these herbicides bind to the D-1 protein, they competitively block the flow of electrons through photosystem II.

Sensitive plant and killed because electron unable to pass through photosystem II increases are transferred through a series of reactions to other reactive toxic compounds. These unstable compounds react with plant cell membranes and cause chloroplast swelling, membrane leakage, and cellular destruction.

Resistance to electron transport inhibiting herbicides in many weed biotypes is nearly always the result of a slight modification in the D-1 protein. Thus, the herbicide is not longer capable of binding to the membrane-bound protein.

Electron acceptors

The bipyridilium herbicides, paraquat and diquat, also affect electron transfer. Rather than block electron transfer, these compounds accept electrons from photosystem I. When the herbicide accepts an electron it becomes reduced to form the paraquat radical. The compound, in turn, reduces molecular oxygen to form toxic free radicals, including superoxide, hydroxyl radical and singlet oxygen. Although all these compounds cause cellular damage, hydroxyl radical is very reactive and rapidly destroys unsaturated lipids, including membrane fatty acids and chlorophyll. This results in membrane destruction, rapid membrane destruction, foliar wilting and a waterlogged appearance, and tissue necrosis. Since paraquat is converted back to its herbicidal form after transferring an electron to superoxide, it can act catalytically to continuously generate toxic oxygen molecules.

Bleaching Agents (Pigment Inhibitors)

Pigment inhibitors block the synthesis of carotenoids. The symptoms they exhibit include a bleaching of the stem and foliar tissues. This occurs because chlorophyll is susceptible to photo-oxidation in the absence of protective carotenoids. Fluridone, norflurazon, amitrole, clomazone inhibit an enzyme within the biosynthetic pathway of carotenoids. Fluridone and norflurazon inhibit the activity of phytoene desaturase in the chloroplast thylakoids. Amitrole appears to block the activity of another enzyme, zeta-carotene desaturase, with occurs further down the carotenoid synthesis pathway. Clomazone also inhibits carotenoid synthesis, but its specific site of action has not yet been identified.

The diphenylether herbicides also act on the pigment biosynthesis pathway. However, the primary action of these compounds is the rapid production of phytotoxic free radicals. Consequently they are classified as herbicides which rapidly destroy cell membranes.

Lipid Synthesis Inhibitors (ACCase)

The aryloxy phenoxy propionate and cyclohexanedione herbicides are postemergence graminicides which inhibit lipid synthesis. The primary target is acetyl-coenzyme A carboxylase (ACCase), a plastid-localized enzyme that catalyzes the conversion of acetyl-CoA to malonyl Co-A. This reaction is one of the initial steps in the formation of fatty acids. Broadleaf species have an altered binding site that is naturally insensitive to these herbicides. In grasses, inhibition in fatty acid synthesis blocks the production of phospholipids used in building new membranes. Under rapid growing conditions, membrane synthesis cannot keep up with cell enlargement processes. This results in cellular destruction, generally in the growing points where cell expansion is greatest.

Many weed biotypes have developed resistance to the aryloxy phenoxy propionate and cyclohexanedione herbicides. This appears to be due to either enhanced metabolism of the herbicide or an altered ACCase.

Cell Wall Synthesis Inhibitors

Cell walls are a complex matrix consisting of predominantly cellulose, but also hemicellulose, pectin, and structural glycoproteins. Primary cell wall synthesis is most rapid in elongating cells of near meristematic regions. Dichlobenil and isoxaben inhibit cellulose synthesis in susceptible plants. Although quinclorac also inhibits cell wall synthesis, it appear to inhibit both cellulose and hemicellulose. This suggests that the target site of quinclorac is different than dichlobenil and isoxaben. With all three herbicides, cell wall injury is first characterized by necrotic bands near the zones of elongation in shoots and roots.

Cell Division Inhibitors

All growth in any organism must be preceded by cell division. This process is not uniformly distributed in plants. It is restricted to certain zones called meristems. The principle meristematic zones are in the root tips, shoot tips, vascular cambium, and just above the nodes in grasses and other monocots. Cell division alone does not cause increased size, but the cellular products of division do cause growth.

Cell cycle inhibitors

Cell division consists of two primary phases, interphase and mitosis, which make up the cell cycle. Dividing cells spend considerably more time in interphase where 1) proteins are synthesized

in preparation for DNA synthesis, 2) chromosomes are replicated through DNA synthesis, and 3) additional proteins are synthesized in preparation for mitosis. Some herbicides, such as napropamide, may inhibit cell division by blocking the synthesis of proteins either before or after DNA synthesis. No specific site of action has yet been identified for any cell division inhibitor, except for some of the microtubule assembly inhibitors. Furthermore, there is no one symptom which is characteristic of the cell cycle inhibitors. Applied preemergence, some of these herbicides inhibit germination, other inhibit emergence. In other cases the plants may be very stunted or the meristematic region may die and become necrotic. Applied postemergence, stunting and death of the growing point is also common.

Microtubule Assembly Inhibitors

Mitosis is the process by which duplicated pairs of chromosomes separate and form a new daughter cell. It can be divided into four stages; prophase, metaphase, anaphase, and telophase.

One of the key proteins involved in mitosis is tubulin. Tubulin subunits polymerize non-enzymatically to form long hollow structures called microtubules. At the same time the other end of the microtubule is depolymerizing and releasing the tubulin subunits. When the rate of polymerization is occurring more rapidly than the depolymerization process, microtubules are formed. One of the primary functions of microtubules is to align the chromosomes at the equatorial poles of the dividing cell in metaphase. This structure, called the spindle apparatus, also facilitates the movement of chromosomes to either end of the cell during anaphase. Microtubules also function in other important capacities; they 1) determine the plane of cell division during prophase, 2) play a critical role in cell plate formation during

telophase, and 3) are involved in spiral orientation of cellulose microfibril deposition and thus regulate cell wall formation and the cylindrical, elongated cell shape.

Dinitroaniline herbicides bind to tubulin at the polymerizing end of the microtubule. The binding of these herbicides prevents further assembly of microtubules, but has no effect on disassembly of the tubule on the other end. This leads to the disappearance of microtubules from the cell. Although prophase may appear normal, chromosomes are unable to align at the metaphase plate or migrate to their respective poles. Furthermore cell wall formation does not occur at telophase. The loss of microtubules not only inhibits cell division, but results in abnormal randomly oriented microfibril (cell wall) deposition. Consequently, cells in the elongation zone do not assume a tubular shape but appear more spherical and swollen. This accounts for the root tip swelling characteristic of the dinitroaniline herbicides.

Besides the dinitroanilines, other herbicides also interfere with microtubule assembly. Pronamide also appears to bind to tubulin, but only partially prevents microtubular formation. With undeveloped microtubules, chromosome alignment and separation cannot be completed. Although the specific site of action of DCPA is not known, it disrupts cell wall formation. This may be due to an inhibitor affect on microtubule function as well as microtubule formation. Dithiopyr and thiazopyr also cause disruption of mitosis and symptoms similar to the dinitroanilines, but do not appear to bind to tubulin. It has been proposed that these herbicides alter microtubule assembly and disassembly by interacting with the microtubule associated proteins or the microtubule organizing centers.

Amino Acid Inhibitors

Acetolactate Synthase (ALS) Inhibitors

Although the sulfonylurea, imidazolinone, triazolopyrimidine, and pyrimidinyloxybenzoate herbicides can be structurally quite different, they are all highly active compounds which inhibit the activity of acetolactate synthase (ALS), also called acetohydroxyacid synthase (AHAS). ALS catalyzes the first step in the synthesis of the branched-chain amino acids valine, leucine and isoleucine. Although cell division and plant growth can he inhibited within a couple of hours of herbicide treatment, death occurs slowly. The rate of death is probably linked to the total pool size of the branched-chain amino acids available to the plant tissues. Thus, older plants with large amino acid reserves will survive longer than younger plants.

Numerous weed species have developed resistance to the ALS inhibitors. In some cases, this is due to an enhanced ability to metabolize the herbicide to non-phytotoxic constituents. In most cases, however, resistance is due expression of an altered ALS insensitive to these herbicides.

EPSP Synthase Inhibitors

Sulfosate is the trimethylsulfonium salt of glyphosate, and thus its mode of action is identical. Glyphosate inhibits the synthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan. Its activity is specific to the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in the shikimic acid pathway. These essential amino acids are important for protein synthesis and other biosynthetic pathways critical to growth. However, it has been postulated that, in addition to depletion of aromatic amino acids, the herbicidal action of glyphosate may also be due to unregulated accumulation of shikimate. The role that shikimate plays in the eventual death of treated plants is still unclear.

Glyphosate also interferes with normal carbohydrate translocation in plants. It has been demonstrated to inhibit the import of sucrose to sink leaves, the export of sucrose from source leaves, and the net starch accumulation in source leaves. This disruption in carbon metabolism would eventually starve the plant of compounds required for growth. The appearance of a red coloration on leaves and stem of treated plants is due to the accumulation of the pigment anthocyanin. Anthocyanin accumulation is a typically symptom of an interference in carbon metabolism.

Despite the wide use of glyphosate around the world, only one species in Australia [rigid or annual ryegrass (*Lolium rigidum*)]. The mechanism of resistance, in this case has not been determined.

Energy Production Inhibitors

The symptomology and growth responses associated with application of the thiocarbamate and chloracetamide herbicides is very similar. The major symptoms seen in susceptible plants include decreased wax production, an inhibition in cell elongation, and abnormal foliar development. Thus, it is often assumed that they share a similar mode of action. It is clear, however, that these herbicide do not inhibit a single enzyme or metabolic process. They have been reported to inhibit the synthesis of lipids, fatty acids, leaf waxes, terpenes, flavonoids, gibberellin, and proteins. In addition, they also interfere with cell division and hormone regulation. Although protein synthesis is not the primary site of action, it appears that the mechanism of action may involve alkylation of enzymes with sulfhydryl groups. The major sites include lipoic acid, an acyl carrier protein, and coenzyme A. Because these herbicide do not have a specific target site, it is unlikely that weed resistance will develop due to an altered site of action.

Little is known of the mode of action of bensulide. It has been shown to inhibit root elongation and cell division and other metabolic processes. Ethofumesate is thought to inhibit long chain fatty acid synthesis, although the specific site of action is unknown.

Cell Membrane Destruction

Herbicides can damage cell membranes by generating excessive levels of free radicals (toxic oxygen molecules), inhibiting glutamine synthetase, or by directly affecting membrane integrity.

Free radical generators

The mechanism of free radical generation by paraquat and diquat have previously been discussed. Other herbicides, including the diphenylethers and oxadiazon, also cause membrane damage through the excessive accumulation of toxic oxygen molecules. The primary target site of the diphenylether herbicides and oxadiazon is protoporphyrinogen oxidase (Protox), an enzyme involved in the biosynthesis pathway of chlorophyll and heme. An inhibition in Protox by these herbicides leads to the accumulation of protoporphyrinogen IX. When this compound diffuses out of the thylakoid membranes it is oxidized to form protoporphyrin IX (PPIX). Because this compound accumulates outside of its normal environment, it is not further metabolized as it would be in the thylakoid membranes. Light absorbed by PPIX causes the release of electrons to oxygen molecules to form singlet oxygen and other free radicals. This produces a chain reaction which rapidly results in lipid peroxidation and membrane destruction similar to that of the bipyridilium herbicides.

Inhibition of glutamine synthetase

Glufosinate inhibits glutamine synthetase, which is the initial enzyme in the pathway that assimilates inorganic nitrogen (ammonia) and the amino acid glutamate into glutamine. By blocking this reaction, ammonia levels increase by as much as 100 times higher than in untreated tissues within a day. This process occurs much faster in the presence of light. Excessive ammonia concentration dissipates the pH gradient across the membrane, thus alters the ability of membranes to function properly. This can lead to an uncoupling of photosynthetic electron transport in plant cells. Although glufosinate primarily inhibits amino acid and nitrogen metabolism, its toxic effect is to disrupt cell membranes. Glufosinate injury occurs slower than other cell membrane disruptors, but symptoms resemble those of paraquat or the diphenylethers herbicides.

Direct effect on membranes

Both MSMA and pelargonic acid cause rapid cellular desiccation by cell membranes destruction. The specific effect of these herbicides on membranes is not well understood. With pelargonic acid, cell destruction occurs very rapidly, and symptoms can be observed within minutes of treatment.

General Cell Toxicants

Acrolein appears to act as general cell toxicants that reacts with sulfhydryl groups associated with many protein and macromolecules. Sodium chlorate acts as a strong oxidizing agent in plants and also blocks protein sulfation. Because of their general affect, both compounds disrupt the activity of a number of enzymes and interfere with several plant metabolic processes.

Herbicides With Unknown Mode of Action

The mode of action of nearly all recently developed herbicides are well known. However, little is known of the mode of action of a number of older herbicides, particularly inorganic compounds and organic arsenicals. These herbicide typically disrupt of a number of metabolic processes, but no specific site of action has yet been identified.