Chapter 11

MODES OF ACTION OF HERBICIDES USED IN COTTON

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INTRODUCTION

WHY UNDERSTAND MODE OF ACTION?

Weed scientists, pesticide chemists and those interested in pest management have become increasingly interested in learning how herbicides kill target plants. Their reasons and motives for wanting this information are varied and sometimes overlap.

Scientists involved in herbicide discovery have made mode of action studies an integral part of several of their strategies for finding new herbicides. One approach involving mode of action is to design herbicides to inhibit particular biochemical processes. This is attractive to industry because molecular sites unique to higher plants can be chosen, thus reducing potential animal toxicological problems. Also, an inhibitor of a particular enzyme might be a good selective herbicide because it differs between target species and associated crops. Knowledge of structures of substrates, products, cofactors and already known inhibitors of the enzyme gives the chemist a start in the design of the herbicide.

Another use of mode of action information is in intelligently improving herbicides by structure-activity studies. Knowledge of the molecular site of action provides a primary assay for doing structure-activity studies. For example, to improve a herbicide that kills the plant by inhibiting a particular enzyme, a large number of analogues could be screened by simply determining their ability to inhibit the enzyme in a test tube. This method is generally much more rapid and less expensive than are bioassays.

The pesticide industry is also becoming more interested in knowing the molecular site of action of its herbicides. This information can be used to defend or challenge patents and to defend or challenge toxicological studies. A structural analogue that hits an entirely different molecular site for its herbicidal action than a somewhat similar molecule might have a better chance in court to be ruled a different and unique chemistry not covered by a competitor's patent. Also, a compound known to kill plants by inhibiting an enzyme found only in plants might have fewer problems in toxicological studies than a herbicide that kills

plants by inhibiting processes common to plants and animals such as mitochondrial respiration or potassium transport.

Knowing the mechanism of action of different herbicides is also useful in predicting possible herbicide interactions. For instance, a herbicide that causes rapid cellular collapse is likely to antagonize one that must be translocated to meristematic areas for activity. Thus, paraquat (Gramoxone®), a rapidly-acting contact herbicide that depends on photosynthesis, would be likely to antagonize glyphosate (Roundup®), a herbicide that must be translocated to meristems for maximal effect. However, bentazon (Basagran®), a photosynthesis inhibitor, will increase weed control by paraquat by giving paraquat time to translocate before it becomes lethal at the cellular level. In this manner, design of synergists or protectants can be based on knowledge of herbicide mode of action.

Absorption and translocation are parts of the mode of action of a herbicide. Understanding these processes for a particular herbicide is also important in predicting interaction of the herbicide with other pesticides, additives and adjuvants. Understanding the influence of the environment on herbicide efficacy is particularly dependent on knowing the entire mode of action of a herbicide, especially its absorption and translocation characteristics.

Determining the mechanisms of resistance of weeds to herbicides is becoming more important because the incidence of herbicide resistance is increasing geometrically (LeBaron and Gressel, 1982; Gressel, 1986). Understanding the nature of resistance is dependent on knowing the mode of action of the herbicide to which resistance has developed. This knowledge offers an opportunity to intelligently design an alternative herbicide that affects the same site or to take advantage of the genetic alteration in a way that might put the resistant mutant at a disadvantage. Additionally, the altered gene that confers resistance might be transferred to susceptible crops by new methods of genetic engineering (see next to last section).

LEVELS OF MODE OF ACTION

The mode of action of a herbicide is a complicated and multifaceted process, beginning with the interaction of the herbicide with the plant surface and ending with cellular death. The site at which the herbicide affects the plant can be considered on an organ (*e.g.*, root or leaf), tissue (*e.g.*, mesophyll or meristem), cellular, organelle (*e.g.*, mitochondria or chloroplast) or molecular basis. In addition, at each level there are secondary and tertiary effects. For example, secondary effects of blocking electron transport in photosynthesis are blocked ATP and NADPH synthesis, which in turn, halts carbon dioxide fixation. This results in reduced synthesis of sugars which ultimately reduce the synthesis of everything else in the plant. Simultaneous with the reduced synthetic capabilities caused by the photosynthetic inhibitor are destructive secondary and tertiary effects of unchannelled energy dissipation from light-energized chlorophyll molecules that cannot lose their energy through normal photosynthetic processes.

The effects of these secondary molecular processes can be examined and described at each level of action. Additionally, there are sometimes more than one direct molecular site of action of a herbicide, although the phytotoxicity is generally almost exclusively due to only one of the molecular sites of activity. For instance, a herbicide might inhibit several enzymes, but most of the herbicidal activity is usually associated with only one inhibitory activity. In cases where there are more than one direct or "primary" molecular site of action of a herbicide, the relative importance of the different primary sites may vary between species. Thus, a full and detailed description of how a herbicide kills a plant is enormously complex.

Furthermore, herbicides often have non-toxic effects at low dosages on target species or at low or high dosages on plant species that are resistant to them. For instance, low dosages of triazine herbicides (*e.g.*, Caparol®, AAtrex®) can enhance nitrogen metabolism in some species (Ries *et al.*, 1967) and sublethal levels of many herbicides can induce synthesis of certain secondary products (Duke, 1985b). Few of these effects have been well characterized.

The level at which the mechanism of action is most clear is usually at the primary molecular level. For clarity and brevity, this is the level at which emphasis will be placed in this chapter. Sources of more detailed information on the mechanisms of action of herbicides include Duke (1985a), Fedtke (1982), Moreland *et al.* (1982), and Ashton and Crafts (1981).

MODES OF ACTION OF HERBICIDE CLASSES

Only those herbicide classes that are currently being evaluated for use in cotton in the southern United States (York, 1987) or those that have been used over the past decade in the United States (McWhorter, 1988) are discussed.

ARSENICALS

Two arsenic compounds, DSMA (disodium methanearsonate) and MSMA (monosodium methanearsonate), are commonly used in cotton weed control. Other forms of arsenic such as arsenite, arsenate and cacodylate are more phytotoxic than methanearsonate (Sachs and Michaels, 1971); however, methanearsonate salts are superior herbicides because they give less contact damage and are rapidly translocated through the plant symplast (the living part of the plant) (Wauchope, 1983). Methanearsonate translocation is much like translocation of phosphate. Transport is toward growing areas or storage areas, making it effective against perennial weeds that have underground storage organs.

Since little or no carbon-arsenic bond breakage occurs in plants (Domir *et al.*, 1976), the methanearsonate ion is probably the toxic species of MSMA and DSMA. Its mode of action at the molecular level is not well understood. Effects on amino acid content, respiration and chlorophyll synthesis have been reported (Wauchope, 1983), however, none of these effects appear to be primary.

A primary effect that may well explain the herbicidal activity of methanearsonate was proposed by Knowles and Benson (1983). They demonstrated that methanearsonate can be photochemically reduced by photosystem I of photosynthesis to form sulfhydryl group reagents, including arsenomethane (Figure 1). Arsenomethane reacts with sulfhydryl groups of enzymes involved in carbon fixation and its regulation, inhibiting carbon fixation. This is especially apparent in the C4 plant johnsongrass. C4 plants like johnsongrass assimilate carbon (C) through the C4 pathway and are considered to be more efficient in photosynthesis than other plants. In johnsongrass methanearsonate causes rapid buildup of malic acid due to inhibition of $NADP^+$-malic enzyme, a C4 enzyme especially sensitive to reagents that react with sulfhydryl groups. The elimination of carbon fixation in bright light causes rapid photooxidative damage (leaf burning) due to uncontrolled dissipation of absorped light energy by several mechanisms (Halliwell, 1985).

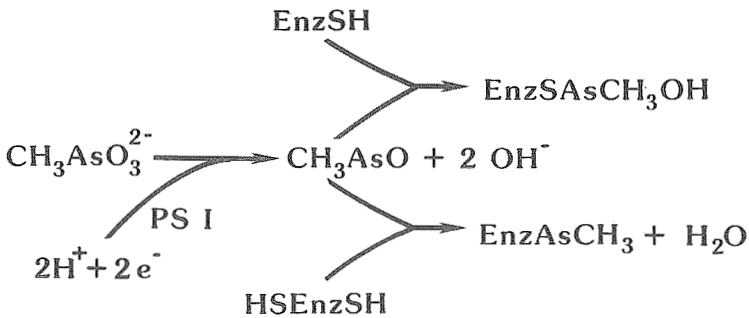

Figure 1. Proposed mode of action of arsenomethane herbicides. PSI = photosystem I; $CH_3AsO_3^{2-}$ = methanearsonate; CH_3AsO = arsenomethane; EnzSH = sulfhydryl-containing enzyme; HSEnzSH = enzyme with two adjacent sulfhydryl groups.

The basis of selectivity for the methanearsonates may be differential conjugation in some cases. Several studies have shown that MSMA is rapidly bound to sugars, amino acid and organic acids in plants. Sckerl and Frans (1969) found that methanearsonate was conjugated to a ninhydrin-positive molecule in sensitive johnsongrass but not in tolerant cotton. Thus, the molecule may be bioacti-

vated to a more toxic or more translocatable species in order to be herbicidally effective. In other cases, differential absorption appears to explain differences in susceptibility. For instance, Keeley and Thulen (1971) found good correlations between control of yellow and purple nutsedge with DSMA and MSMA and absorption of the herbicides.

ARYLOXYPHENOXY ALKANOIC ACIDS

The aryloxyphenoxy alkanoic acids are a new group of grass-killing herbicides characterized by an alkanoic acid (generally propanoic acid) with an aryloxyphenoxy group attached to an asymmetric carbon. They are members of a larger class of herbicides, the polycyclic alkanoic acids, which also includes the benzoyl-N-phenyl phenoxy alkanoic acids (Duke and Kenyon, 1988). The polycyclic alkanoic acids all appear to have the same mechanism of action; however, only the aryloxyphenoxy alkanoic acids of this group are utilized in cotton. All members of this group are sold as propanoic acid esters—usually methyl, ethyl, or butyl.

Commonly used members of this group that are used in cotton are fluazifop-butyl (Fusilade®) and haloxyfop-methyl (Verdict®). Although diclofop-methyl (Hoelon®) is not commonly used in cotton, virtually all studies of the mechanism of action of this group of herbicides have been conducted with diclofop. Since the symptomologies of all members of this herbicide group are the same, there is little reason to expect that there are any significant differences in the mechanisms of action of the members of this class of herbicides.

This group of herbicides is readily absorbed by plant leaf cuticles. The ester group apparently increases absorption by increasing lipophilicity. Thus, members of this herbicide group probably cross the plant cuticle by becoming incorporated into the waxy matrix of the cuticle and then diffusing into adjacent cells by movement into membranes. As a result of its ability to rapidly move into the lipophilic domains of the cuticle, this group of herbicides is relatively "rainfast." Soon after entry into the cell, or perhaps even in the cell wall, the ester linkage is broken by hydrolysis. Hydrolysis bioactivates the herbicide because the ester form of the herbicide is not active at the site of action.

Despite the efficient absorption of members of this herbicide group, they generally are translocated rather poorly (Duke and Kenyon, 1988). However, there are exceptions such as fluazifop-butyl which has been reported to translocate relatively well (translocation of 25 to 60 per cent of absorbed) (Kells *et al.*, 1984; Wills and McWhorter, 1983; Nalewaja *et al.*, 1986). The translocated form of the herbicide has been determined to be almost exclusively the de-esterified form (Carr *et al.*, 1986). Generally, the more lipophilic a compound, the less phloem-mobile it is.

Herbicidal injury by these herbicides usually follows the pattern of: (a) cessation of growth, (b) chlorosis, (c) meristematic necrosis and, ultimately, (d) death and necrosis of the remainder of the plant. Although many effects of this herbi-

cide group on leaf tissues have been described, the tissues that appear initially to be most drastically affected are meristems. Intercalary meristems, the basal meristems of grass leaves, are particulary vulnerable since these poorly translocated herbicides accumulate most rapidly at this meristem as they move basipetally from the leaf blade. No microscopic studies have been published on intercalary meristem effects of these herbicides; however, Vaughn [see Duke and Kenyon (1988)] showed that halxoyfop-methyl causes extensive vacuolization and vacuolar engulfment of cytoplasm in root meristems of grain sorghum [*Sorghum bicolor* (L.) Moench].

Some studies have indicated that this class of herbicides act as auxin antagonists because artificial auxins such as 2,4-D could reverse their effects. However, structure-activity studies (Taylor *et al.*, 1983) have indicated that the antagonism of phenoxy propanoic acid-type herbicides such as 2,4-D, and aryloxyphenoxy alkanoic acids is due to structural similarities and not to the auxin-like activity of the phenoxy propanoic acids.

The most definitive studies to determine the site of action of these compounds all indicate that the primary site of action is lipid synthesis. Hoppe (1985) found diclofop to inhibit fatty acid synthesis with an I_{50} of 0.1 μM after 0.5 to 4 hours of pretreatment in sensitive species, but not in insensitive (tolerant) species. Diclofop (Hoelon®) and other aryloxyphenoxy propanoic acid herbicides stopped fatty acid synthesis within one hour in isolated chloroplasts of sensitive maize, but not in insensitive bean (Hoppe and Zacher, 1985) (Figure 2). These studies were done with green chloroplasts because the proplastids of meristems are virtually impossible to isolate. However, the immature, rapidly metabolizing plastids of the intercalary meristem undoubtedly are sites of even more active lipid synthesis than mature chloroplasts. Thus, one might expect the cellular results of inhibition of lipid synthesis of the intercalary meristem, and cells rapidly proliferating from it, to be, as observed, general membrane disruption and cellular collapse.

More recent work from several laboratories has shown that the primary molecular site of action of this herbicide class is acetyl-CoA carboxylase, an enzyme required in lipid synthesis (Burton *et al.*, 1987; Secor and Cséke, 1988; Kobek *et al.*, 1988). The enzyme isolated from grass species such as barley is sensitive to these herbicides at very low levels.

There is no good evidence that the selectivity of this group of herbicides is based on differential absorption or translocation (Duke and Kenyon, 1988). From recent findings, one would predict that the mechanisms of tolerance or resistance to this group of herbicides resides at the site of action; however, Hoppe (1985) and others (Shimabukuro, 1985) have evidence that metabolic degradation may play a role in the tolerance of some species to these herbicides.

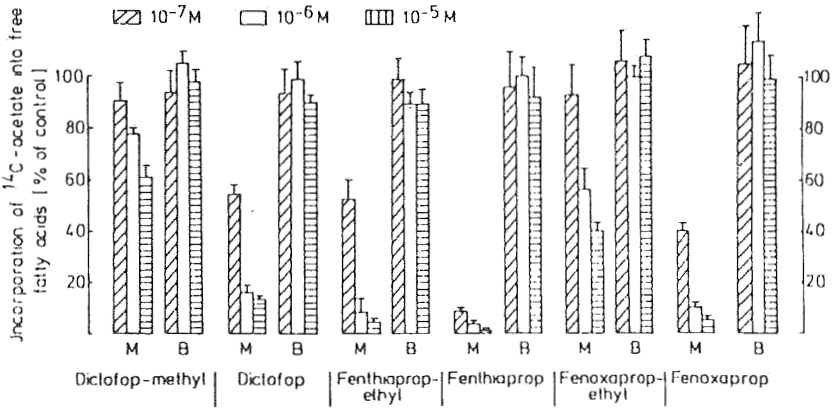

Figure 2. Effect of polycyclic alkanoic acid herbicides on the incorporation of ^{14}C-acetate into free fatty acids by isolated sensitive maize (M) and insensitive bean (B) chloroplasts. Incubation was terminated after 1 hour by lipid extraction. (Hoppe and Zacher, 1985.)

CHLORINATED ALIPHATIC ACIDS

There are two commonly used chlorinated aliphatic acid herbicides, TCA (trichloroacetic acid) and dalapon (2,2-dichloropropionic acid). Of these, only dalapon (Dowpon®) is currently used in cotton. Dalapon is a postemergence herbicide and symptoms of herbicide injury with this herbicide are growth inhibition, chlorosis and distortion of growth patterns. It is readily absorbed by foliage and is translocated both apoplastically (outside of the cytoplasm) and symplastically (from cell to cell, within the cytoplasm) (Ashton and Crafts, 1981). Dalapon is not readily metabolized in plants, but is readily degraded in the soil (Foy, 1975). Even in cotton, only about 10 per cent of dalapon applied to the plant was degraded during a 10-week period after treatment (Foy, 1961).

The mechanism of action of dalapon at a fundamental level is unknown. It has been reported to be an inhibitor of lipid synthesis, a disrupter of nitrogen metabolism and an inducer of phenolic metabolism (Ashton and Crafts, 1981). There is no good evidence that any of these are primary effects and in some cases conflicting results have been obtained.

Anderson *et al.* (1962) hypothesized that dalapon acts by disrupting nitrogen metabolism, causing toxic levels of ammonia to build up. Later work demonstrated that dalapon altered free amino acid levels in susceptible varieties of plants with no effects on levels in dalapon-resistant varieties. This led to the

hypothesis that dalapon inhibits aminating enzymes involved in amino acid biosynthesis (detailed by Ashton and Crafts, 1981). However, the symptoms of dalapon injury do not resemble those of herbicides such as glufosinate (Ignite®) which have been proven to kill by causing massive ammonia buildup by inhibiting the aminating enzyme, glutamate synthetase (Wild *et al.*, 1987).

The only proven primary site of action is the effect of dalapon on the synthesis of pantothenic acid in microorganisms (Hilton *et al.*, 1959; Van Oorschot and Hilton, 1963). Pantothenic acid is required for synthesis of coenzyme A which is required for many metabolic and biosynthetic functions. Hilton *et al.* (1959) found that dalapon competes with pantenoate for a site on the pantothenic acid-synthesizing enzyme. If the herbicide effectively inhibits the same enzyme in plants, this one effect could account for many of the observed secondary effects. Pantothenate fed to dalapon-affected barley plants will partially overcome the effects on abnormal tillering, however, little or no reversal of growth effects is seen (Hilton *et al.*, 1959). Since these findings, little support has been garnered for the hypothesis that inhibition of pantothenic acid is the primary mechanism of action of dalapon. In fact, the literature is filled with conflicting evidence. No clear picture emerges.

Dalapon will inhibit mineral absorption by excised roots within 20 minutes at only 0.1 mM (Ferrari *et al.*, 1981). Furthermore, there is a good correlation between the magnitude of this effect and the phytotoxicity of dalapon to the tested species. The only other physiological parameter that has been correlated with susceptibility to dalapon is higher levels of the amino acid asparagine in tolerant genotypes of bermudagrass (Sistrunk, 1969).

CHLOROACETAMIDES

The chloroacetamides are represented by the herbicides alachlor (Lasso®) and metolachlor (Dual®). The mechanism of action of this group of herbicides is still unknown. Both alachlor (Sharp, 1988) and metolachlor (LeBaron *et al.*, 1988) have recently been reviewed. These compounds are generally absorbed readily and apoplastically translocated (translocated outside of the cytoplasm). At the whole plant level, chloroacetamides are growth inhibitors, greatly inhibiting both germination and growth.

Many secondary effects of these compounds have been found. Although many studies have shown that protein synthesis is inhibited by chloroacetamides, *in vitro* studies demonstrated that there is no direct effect on plant protein synthesis (Deal *et al.*, 1980). Only a few distinct effects that may be closely related to the primary mechanism of action have been described. These compounds are known to alkylate proteins (McFarland and Hess, 1986). The alkylation varies with the specific protein and generally herbicidal activity correlates with alkylation potential. Their herbicidal activities may be associated with alkylation of specific proteins. Weisshaar and Böger (1987) have shown that metalachlor has a very pronounced and rapid inhibition (76 per cent in 4 hours) of ^{14}C-acetate incorpo-

ration into certain acyl lipids (monogalactosyl diacylglycerol) in the green alga *Scenedesmus acutus*. This process and others greatly inhibited by chloroacetamides, such as anthocyanin synthesis (Molin *et al.*, 1986), require coenzyme A. Chloroacetamides can alkylate coenzyme A *in vitro* (Leavitt and Penner, 1979). Thus, although these herbicides may inhibit several enzymes by alkylation, those processes dependent on coenzyme A may be the most sensitive.

Rapid effects of this herbicide group on membrane leakage, mineral ion uptake and amino acid uptake have also been reported (Balke, 1985). However, the symptomology of phytotoxicity at the whole plant level (severe growth inhibition) is not characteristic of compounds that rapidly destroy membrane function.

CINEOLE DERIVATIVES

Cinmethylin (Cinch®) is a preemergence, cineole-based herbicide that is registered for control of grasses in cotton. This herbicide is closely related to 1,8-cineole, a sesquiterpenoid allelochemical produced by many species, including those of the genera *Salvia* and *Artemisia*. Very little is known of its mechanism of action.

The only symptomology for this herbicide is inhibition of growth. In the only published paper on the mechanism of action of cinmethylin, El-Deek and Hess (1986) reported that there was no effect of the herbicide on cell elongation of oat coleoptiles. They did find, however, that cell division was inhibited, although all stages of mitosis were similarly affected. They theorized that the herbicide prevents entry into mitosis rather than being a mitotic disrupter. Growth inhibition by cinmethylin could not be reversed by root-feeding affected plants with supplemental amino acids, suggesting that the herbicide does not affect amino acid synthesis.

CYCLOHEXANEDIONES

This herbicide group is used postemergence in much the same way that the aryloxyphenoxy propanoic acids (see above) are used. Examples of cyclohexanediones that are used in cotton are sethoxydim (Poast®) and clethodim (Select®). The etiology and symptomology of herbicidal damage caused by this group is virtually identical to that caused by the aryloxyphenoxy propanoic acids; that is, the compound is absorbed by the leaf and translocated to the intercalary meristem where it disrupts meristematic cell plastid development and cellular integrity (Ishikawa *et al.*, 1980; Lichtenthaler and Meier, 1984). As with the aryloxyphenoxy propanoic acids, the primary site of action appears to be inhibition of lipid synthesis. Ishihara *et al.* (1987) reported that sethoxydim inhibits polar lipid synthesis in corn root tips within 15 minutes after treatment. No significant effects on synthesis of proteins, nucleic acids or sugars were measured within two hours. Lichtenthaler *et al.* (1987) and Kobek *et al.* (1988) found cyclohexanediones to strongly inhibit lipid synthesis in the chloroplasts of sensitive

species within 20 minutes, however, no inhibition was detected in chloroplasts of insensitive species (Figure 3).

As with the aryloxyphenoxy propanoic acids such as diclofop (Hoelon®), several laboratories have recently provided good evidence that this herbicide class inhibits lipid synthesis by inhibiting acetyl-CoA carboxylase activity (Burton *et al.*, 1987; Focke and Lichtenthaler, 1987; Rendina and Felts, 1988; Secor and Cséke, 1988). Acetyl-CoA carboxylase activity of insensitive plant species was affected very little at concentrations that severely inhibited the enzyme from susceptible grasses (Burton *et al.*, 1987; Rendina and Felts, 1988).

The mechanism of resistance to cyclohexanediones appears to be at the sight of action. Careful studies (Struve *et al.*, 1987) have found no differences in absorption of sethyoxydim by sensitive [Kentucky bluegrass (*Poa pratensis*) and sheep fescue (*Festuca ovina*)] and resistant [annual bluegrass (*Poa annua*) and red fescue (*Festuca rubra*)] grasses. As mentioned above, lipid synthesis and acetyl-CoA activity of sensitive species, but not insensitive species, are inhibited by this family of herbicides.

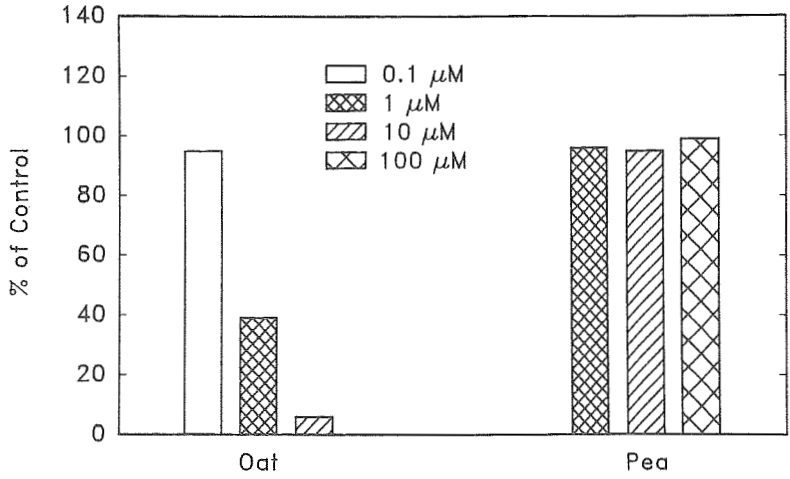

Figure 3. Effects of sethoxydim on incorporation of ^{14}C-acetate into free fatty acids by isolated chloroplasts of oat (sensitive) and pea (insensitive) over a 20 minute period. (Redrawn from Lichtenthaler *et al.*, 1987.)

DCPA

Dimethyl tetrachloroterepthalate (DCPA) is a preplant herbicide that is not absorbed readily by leaves. It neither is translocated well nor is it known to be metabolized by plants. DCPA (Dacthal®) is a growth inhibitor and differentiation

disruptor. It stops cell division in root tips (Bingham, 1968; Chang and Smith, 1972). Cell division is arrested in prophase and aberrant cell nuclei are common in DCPA-affected cells (Holmsen and Hess, 1984). Concomitant with arrested cell division, researchers have observed abnormal cell nuclei, abnormal cell differentiation, and altered cell wall formation (Nishimoto and Warren, 1971; Anderson and Shaybany, 1972; Holmsen and Hess, 1985; Shaybany and Anderson, 1972). As observed by Holmsen and Hess (1984), these are effects that might be expected from a microtuble disrupter. Holmsen and Hess (1985) found DCPA to have effects similar to both colchicine and propham (Chem-Hoe®), both disruptors of mitotic spindle structure and function. Robinson and Herzog (1977) found that DCPA inhibited the polymerization of pig brain tubulin in a test tube, although it had little effect on cell wall organization in the alga *Oocytis solitaria*. Vaughn *et al.* (1987) found DCPA causes loss of spindle and cortical microtubules in both trifluralin-sensitive goosegrass and a trifluralin-resistant goosegrass with altered microtubles (see below). Apparently, if DCPA intereacts directly with tubulin, the protein from which microtubules are constructed, the site of interaction of DCPA differs from that of the dinitroanilines.

DINITROANILINES

A large number of dinitroaniline herbicides, including dinitramine (Cobex®), oryzalin (Surflan®), trifluralin (Treflan®), profluralin (Tolban®), pendimethalin (Prowl®) and fluchoralin (Basalin®) are used in cotton. They are all growth inhibitors used as preplant-incorporated herbicides much like DCPA (see above). Like most preplant herbicides, the dinitroanilines are taken up primarily by roots; however, other subterranean parts of seedlings can be important in uptake. Translocation from roots to shoots or vice versa is generally negligible. The dinitroanilines are extensively metabolized by both microorganisms and higher plants; however, few studies have been designed to differentiate between microbial degradation of the herbicide before it entered the plant and degradation by the plant. Ashton and Crafts (1981) provide a detailed literature review of uptake, translocation and metabolism of dinitroaniline herbicides.

Trifluralin arrests cell division by preventing prophase chromosomes from properly aligning at the metaphase stage of mitosis (Hess, 1987). These effects are usually very pronounced with herbicide applications in the 1 μM range. Chromosomes are aligned during metaphase by the spindle apparatus which is composed of microtubules which, in turn, are composed of the protein tubulin.

At the ultrastructural level, a reduced number or an absence of microtubules is observed in dinitroaniline-treated cells (Jackson and Stetler, 1973; Bartels and Hilton, 1973; Hess and Bayer, 1974). Hess and Bayer (1977) later reported that trifluralin binds to tubulin protein isolated from the green alga *Chlamydomonas*. Oryzalin was found to have a binding ratio or nearly one molecule of herbicide per molecule of tubulin in *in vitro* studies (Strachan and Hess, 1983). It had insignificant binding to denatured tubulin, intact microtubules (polymerized tubulin)

and non-tubulin proteins. These data strongly suggest that dinitroaniline herbicides inhibit growth and cell development by binding to tubulin monomers and preventing their polymerization into the microtubules required for mitotic spindle formation and cell wall deposition.

This conclusion is further supported by the finding by Morejohn *et al.* (1983) that oryzalin prevents taxol-induced polymerization of rose tubulin. Similarly, Vaughn and Vaughan (1990) found that tubulin of dinitroaniline-sensitive goosegrass does not polymerize in the presence of dinitroaniline herbicides. They also found that the polymerization of tubulin from dinitroaniline-resistant goosegrass was unaffected by dinitroaniline herbicides. One of the subunits of tubulin from the resistant goosegrass biotype was found to be somewhat smaller than in the sensitive biotype. Considerable cross resistance with the phosphoric amides (such as amiprophos-methyl), another herbicide group that disrupts cell division, was found by Vaughn *et al.* (1987), supporting the view that the resistance is due to a structural alteration in tubulin, rather than changes in absorption, translocation or metabolism.

The mechanisms of resistance of other species to dinitroaniline herbicides may also be related to tubulin differences. Vaughan and Vaughn (1988) found that carrots are resistant to this class of herbicides and that their tubulin is different from that of sensitive plant species.

Hilton and Christiansen (1972) found an excellent correlation between seed lipid content and the sensitivity of eleven crop and weed species to trifluralin. Apparently, this highly lipophilic herbicide is readily sequestered into lipid bodies, away from its site of action. This may well explain the tolerance of cotton to this herbicide class because its tolerance to pyridazinones, another group of lipophilic herbicides, has been demonstrated to have a similar basis (Strang and Rogers, 1974).

GLYPHOSATE

Glyphosate (Roundup®) is a non-selective herbicide that exerts most, if not all, of its effects by inhibiting the synthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan). Aromatic amino acids are required for protein synthesis. Thus, blockage of synthesis of these essential amino acids slows and then stops protein synthesis, resulting in arrest of growth processes, cellular disorganization, followed by cellular death. Jaworski (1972) first proposed this as the mode of action, based on the finding that supplying certain organisms with exogenous aromatic amino acids would prevent reduction of growth by glyphosate.

Aromatic amino acids are synthesized by the shikimic acid pathway, a biosynthetic pathway peculiar to microorganisms and plants (Figure 4). Amrhein's laboratory found that glyphosate causes massive accumulation of shikimic acid in treated plants (Amrhein *et al.*, 1980). This led him to examine the effects of glyphosate on 5-enolpyruvyl shikimic acid-3-phosphate [EPSP] synthase, the enzyme that coverts shikimate-3-phosphate to 5-enolpyruvyl shikimic acid

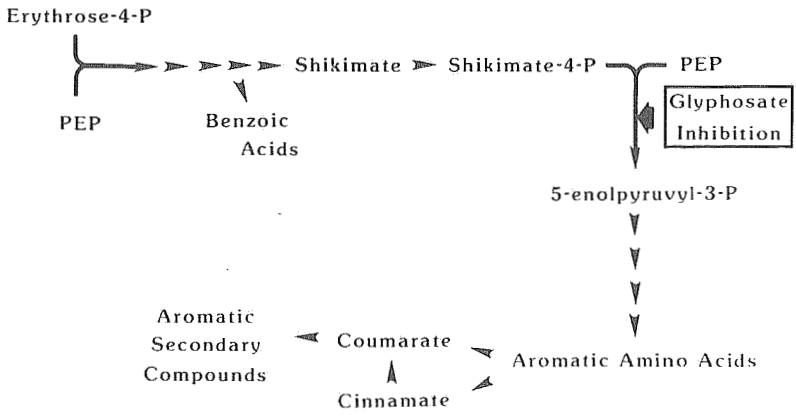

Figure 4. The shikimate pathway with the site of inhibition of glyphosate indicated. PEP = phosphoenolpyruvate.

3-phosphate (Steinrücken and Amrhein, 1980). They found glyphosate to be a potent inhibitor of the enzyme. It since has been found to inhibit this enzyme in a wide variety of plants and microorganisms (Duke, 1988).

Normally, products of the shikimate pathway regulate flow of carbon into this pathway. Inhibition of the pathway causes deregulation of the pathway and more carbon than usual flows into the pathway, removing building blocks from other essential pathways (Kilmer *et al.*, 1981; Jenson, 1986). This eventually disrupts every aspect of plant metabolism. The massive levels of shikimate that accumulate are primarily localized in the plant cell vacuole (Höllander-Czytko and Amrhein, 1983); however, some of this shikimate is used by the plant for synthesis of hydroxybenzoic acids such as protocatechuic, gallic and gentisic acids (Lydon and Duke, 1988a; Cañal *et al.*, 1987).

Glyphosate is used only as a postemergence herbicide because it is inactivated by soil due to its ionic nature. It is an ideal herbicide for foliar application because it is readily absorbed and is efficiently translocated to actively growing tissues of the plant where it accumulates (Duke, 1987; Caseley and Coupland, 1985). Thus, it will kill growing roots, rhizomes, bulbs, tubers and stolons, as well as aerial portions of plants, preventing regrowth, a major problem in controlling perennial weeds.

Glyphosate is a highly water soluble compound. Therefore, it probably moves across the leaf cuticle in hydrated pores. Indeed, absorption of glyphosate is greater in plants with more hydrated cuticles (*e.g.*, in plants under higher relative

humidity or under no water stress). Once the herbicide has crossed the cuticle to move into the solution of the intercellular spaces of the leaf, it moves slowly across the outer plant cell membranes (plasmalemmas) of the photosynthesizing cells (mesophyll) of the leaf and of the phloem cells. Gougler and Geiger (1981) postulated that the low permeability of the plasmalemma for glyphosate allows for efficient long range transport in the plant. The compound enters the phloem in the sprayed leaf, a site of high external glyphosate concentration. More of the compound moves into the phloem than into mesophyll cells because the diffusion gradient is higher across their plasmalemma due the continual movement of the compound to other parts of the plant. Because of the low permeability of the phloem plasmalemma, it does not readily leak back out into the xylem after leaving this site. Thus, its concentration becomes high at terminal sites of phloem transport such as apical meristems and fruits. Because glyphosate is not significantly, if at all, metabolized by higher plants, slow buildup of the herbicide can occur over very long periods of time, eventually resulting in cellular and tissue death.

Because glyphosate is a relatively slow-acting herbicide, the sites of absorption remain metabolically active long enough for considerable phloem transport to occur. There is generally a very high correlation between transport of photosynthate and glyphosate from leaves sprayed with glyphosate (Figure 5).

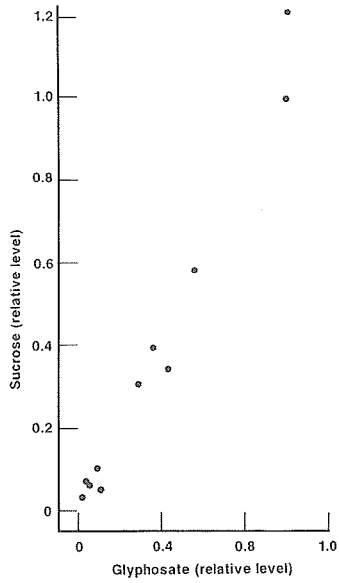

Figure 5. Correlation between sucrose and glyphosate translocation in sugarbeet. (Adapted from Gougler and Geiger, 1981.)

ISOXAZOLIDINONES

The isoxazolidinones are represented by only one commercialized herbicide, clomazone (Command®). This is a relatively new preemergence herbicide that is being tested for use in cotton (York, 1987).

Susceptible species display a loss of chloroplast pigments after treatment, resulting in light green, yellow or entirely white leaves (Duke and Paul, 1986). Unlike other herbicides that inhibit carotenoid synthesis (*e.g.* pyridazinones—see below), this herbicide blocks the synthesis of both carotenoids and chlorophyll by inhibiting the terpenoid pathway at a step early enough to prevent synthesis of geranylgeranyl pyrophosphate (Duke *et al.*, 1985; Duke and Kenyon, 1986b; Sandmann and Böger, 1986). Geranylgeranyl pyrophosphate is required for the synthesis of carotenoids, phytol (an integral part of chlorophyll), and gibberellic acid. Evidence of the loss of all three of these constituents in clomazone-treated plants has been produced. In very sensitive higher plants, there is no synthesis of carotenoids, even in darkness where photobleaching cannot account for the carotenoid loss (Duke *et al.*, 1985). The Shibata shift, a spectral shift caused by attachment of phytol to the chlorophyll chromophore, is prevented by the herbicide (Duke *et al.*, 1985; Duke and Kenyon, 1986b). Furthermore, phytol levels are lowered in algae grown in the presence of clomazone (Sandman and Böger, 1986). Etiolated growth is greatly stunted in treated seedlings (Duke and Paul, 1986; Duke *et al.*, 1985), an indicator of blockage of gibberellic acid synthesis. Sandman and Böger (1986) reversed the growth-inhibiting effect of clomazone on pea seedlings with gibberellic acid. These data all support the hypothesis of Duke *et al.* (1985) that the herbicide blocks the synthesis of geranylgeranyl pyrophosphate (Figure 6).

Sandman and Böger (1987) later demonstrated that clomazone (Command®) does strongly inhibit synthesis of geranylgeranyl pyrophosphate in a cell-free spinach preparation. In this system, they found that there was actually an increase in isopentenyl pyrophosphate, the first product in the terpenoid pathway. With each succeeding step of the terpenoid pathway, they found stronger inhibition. This led them to hypothesize that isopentenyl pyrophosphate isomerase or prenyl transferase is the inhibited enzyme. These enzymes are required for each successive addition of five carbon subunits to simpler terpenoids. They speculate that later steps are more susceptible to the herbicide because of their chloroplast location. Results of Duke *et al.* (1991) show accumulation of sesquiterpenoids in clomazone-treated tissues, indicating that only the conversion of farnesyl pyrophosphate to geranylgeranyl pyrophosphate is inhibited (Figure 6).

Susceptibility differences between soybean (tolerant) and cotton (more susceptible) are not due to uptake or translocation differences (Norman *et al.*, 1990). Similarly, Vencill *et al.* (1990) found no differences in clomazone translocation between soybean, and both susceptible and tolerant pigweed species. They did find higher levels of uptake in susceptible species of pigweed than in a tolerant species. No differences in metabolic products or rates of metabolism were ob-

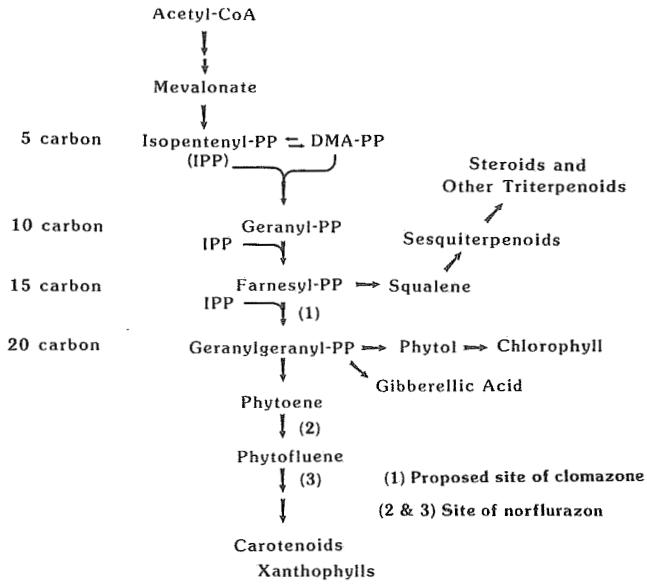

Figure 6. Isoprenoid pathway of terpenoid synthesis in higher plants with the site of carotenoid synthesis inhibition by pyridazinones (norflurazon) and the proposed site of inhibition of the isoxazolidinones (clomazone). PP = pyrophosphate; IPP = isopentenyl pyrophosphate.

served in this study. However, Norman *et al.* (1990) found that susceptible cotton may metabolize clomazone to a herbicidal metabolite (bioactivation). Both studies hypothesized that differences at the molecular site of action may play an important role in explaining tolerance and susceptibility to clomazone.

p-NITROSUBSTITUTED DIPHENYL ETHERS

The *p*-nitrosubstituted diphenyl ethers (NDPE) are foliar-applied contact herbicides that cause rapid chlorosis in light (bleaching) and/or necrosis. Representatives of this herbicide class are acifluorfen (Blazer® or Tackle®), fluorodifen (Preforan®), fomesafen (Reflex® or Frigate®), lactofen (Cobra®) and nitrofen (Tok®).

In general, the NDPE herbicides are poorly absorbed and very poorly translocated. For instance, Ritter and Coble (1981) found only about 4 percent of applied acifluorfen to be absorbed by soybean leaves 48 hours after treatment. Less than one percent of applied acifluorfen was translocated from the treated leaf. In other species, absorption after 48 hours can range from 10 to almost 20 percent, depending on the NDPE (Hawton and Stobbe, 1971a; Ritter and Coble, 1981). However, less than two percent of applied NDPE was reported to be translocated from treated leaves in plants for up to 16 days after treatment (Hawton

and Stobbe, 1971b). Even movement of acifluorfen into cells through the cut edge of a leaf disc is extremely slow (Duke and Kenyon, 1986a).

The NDPEs are readily metabolized by leguminous species. Eastin (1971) found peanuts metabolized 88 percent of absorbed fluorodifen within 72 hours after treatment. Fluorodifen (Rogers, 1971) and acifluorfen (Frear *et al.*, 1983) are rapidly metabolized by soybeans. Detoxification of NDPE herbicides can occur through reduction of the *p*-nitro group to an amide or by cleavage of the ether linkage and subsequent conjugation of the two metabolites with glutathione and sugars (Shimabukuro, 1985).

Much physiological and biochemical information on how these herbicides act is available. Light is an absolute requirement for rapid cellular collapse and/or photobleaching, the herbicidal symptoms seen in target plants in the field (Matsunaka, 1969; Orr and Hess, 1982a). Furthermore, oxygen appears to be necessary for full activity (Orr and Hess, 1982b). Cells without the capacity for photosynthesis, but with green or yellow plastids, are generally more sensitive to these herbicides than photosynthetically active cells (Duke *et al.*, 1984; Duke and Kenyon, 1986; Kenyon and Duke, 1988). This is probably because they cannot regenerate protective reductants (Kenyon and Duke, 1985).

One of the first symptoms of damage by this herbicide group is breakage of the plasmalemma (Orr and Hess, 1982a; Kenyon *et al.*, 1985; Duke and Kenyon, 1987). Visualization of this at the ultrastructural level coincides with detection of electrolyte leakage from affected cells. These symptoms are followed by loss of photosynthetic capacity, loss of chloroplast pigments, and evidence of lipid peroxidation such as ethane evolution and malondialdehyde formation (Kenyon *et al.*, 1985).

The activity of acifluorfen (Blazer®, Tackle®) is virtually temperature independent, after a period of incubation with the herbicide in darkness (Kenyon and Duke, 1988). Thus, the herbicide acts much like a photodynamic dye that produces cellular damage by absorbing light and transferring the absorbed energy to molecular oxygen to form reactive singlet oxygen. Singlet oxygen can initiate lipid peroxidation, resulting in membrane damage and cellular leakage (Halliwell, 1985).

Apparently, the photodynamic dye that causes all of the above effects is protoporphyrin IX, a metabolic precursor of both chrorophyll and heme. Recently, several laboratories have found that NDPE herbicides cause the accumulation of this reddish-brown pigment (Becerril and Duke, 1989; Lydon and Duke, 1988b; Matringe and Scalla, 1988; Witkowski and Halling, 1988). These laboratories showed that inhibitors of synthesis of protoporphyrin IX inhibit the activity of NDPE herbicides almost completely. Protoporphyrinogen oxidase, a pivotal enzyme of the porphyrin synthesis pathway, is strongly inhibited by these herbicides (Matringe *et al.*, 1989a, b; Witkowski and Halling, 1989; Jacobs *et al.*, 1990) and it is now accepted that this enzyme is their molecular site of action in causing protoporphyrin IX to accumulate (Figure 7). Thus, NDPE herbicides

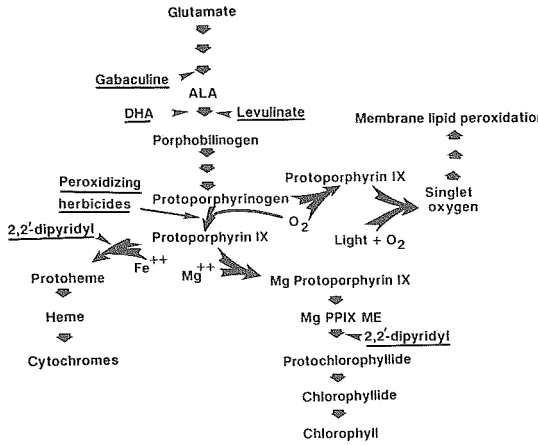

Figure 7. Mechanism of action of NDPE herbicides. NDPE herbicides inhibit the porphyrin synthesis pathway at the point of conversion of protoporphyrinogen to protoporhyrin IX, a photodynamic pigment. Protoporphyrin IX accumulates due to unregulated, non-enzymatic autooxidation of protoporphyrinogen. Gabaculine, dioxoheptanoic acid (DHA) and levulinate inhibit the porphyrin synthesis pathway and also inhibit the activity of NDPE herbicides.

appear to act in much the same way that δ-aminolevulinic acid (ALA) acts—by causing the accumulation of photodynamic tetrapyrole pigments (Rebeiz *et al.*, 1984). In the presence of light and molecular oxygen, these pigments generate highly destructive singlet oxygen which causes all of the subsequently observed effects.

The mechanisms of tolerance to NDPE herbicides are complex. There is no evidence that differences at a primary site of action can account for differences in sensitivity. As mentioned above, leguminous plants rapidly metabolize the NDPE herbicides and are generally tolerant to these herbicides. Less tolerant species such as rape, redroot pigweed, and green foxtail (Hawton and Stobbe, 1971b), cucumber (Eastin, 1972), and common ragweed and common cocklebur (Ritter and Coble, 1981) metabolize these herbicides more slowly than leguminous species. However, absorption of acifluorfen (Blazer®, Tackle®) by ragweed and cocklebur was less than by soybeans (Ritter and Coble, 1981). In studies with rape, redroot pigweed and green foxtail, Hawton and Stobbe (1971a) found that there was no correlation between absorption of and sensitivity to nitrofen (Tok®). The capacity of a species to detoxify toxic oxygen species or free radicals

also may be important in explaining differential sensitivity to NDPE herbicides. Species with ratios of ascorbic acid (vitamin C) to α-tocopherol (vitamin E) in the range of 10 to 15 are considerably more tolerant to oxyfluorfen (Goal®) than species with lower or higher ratios (Finckh and Kunert, 1985). Both ascorbate and α-tocopherol are involved in protection of the plant from toxic radicals and oxygen species (Halliwell, 1985).

PYRIDAZINONES

The only pyridazinone herbicide used in cotton is norflurazon (Zorial®). It is used extensively as a preemergence herbicide. Cotton is tolerant, but not resistant to this herbicide. Cotton is apparently less sensitive to this herbicide than other plant species because it sequesters this lipophilic herbicide into lysigenous glands that are filled with lipophilic terpenoids (Strang and Rogers, 1974). Cotton varieties without these glands are more sensitive to norflurazon than those with glands (Stegink and Vaughn, 1988). Cotton absorbs and translocates norflurazon equally well as sicklepod and corn, both of which are much more sensitive to the herbicide than cotton (Mersie and Singh, 1987). Also, cotton does not metabolize norflurazon at a greater rate than either of these two more sensitive species.

The mechanism of action of norflurazon (Zorial®) is well understood. It inhibits two enzymatic steps in carotenoid synthesis, the desaturation of phytoene and phytofluene, resulting in the accumulation of both of these precursors (summarized by Duke, 1985b and Ridley, 1982) (Figure 6). The symptoms are similar to those caused by clomazone (Command®) (see isoxazolidinones section above) since both herbicides cause total loss of chlorophyll pigments, resulting in a white plant. However, loss of chlorophyll in norflurazon-treated plants is due to the indirect effect of chlorophyll pigments not being protected from photooxidation by carotenoids. Loss of carotenoids also causes photooxidative destruction of other plastid (chloroplasts are green plastids) components. Thus, the seedling is killed by both photooxidative damage and lack of production of photosynthate.

This herbicide is not very effective on green tissue and is thus not used as a postemergence herbicide. The physiological basis for this may be related to the fact that the target enzymes are nuclear-coded and must move from the cytoplasm into the plastid before becoming active in carotenoid synthesis. Findings by Ridley and Ridley (1979) indicate that norflurazon does not enter the plastid of treated tissues and the target enzyme is protected after becoming a functional plastid enzyme. This agrees with the finding that nuclear-coded protein synthesis is required for pyridazinone inhibition of carotenoid synthesis (Grumbach and Drollinger, 1980).

SULFONYLUREAS

The commercialized sulfonylurea herbicides include chlorsulfuron (Glean®), metsulfuron methyl (Ally®), chlorimuron ethyl (Classic®) and sulfometuron methyl (Oust®). None of these are used in cotton because cotton is sensitive to

them. However, an experimental sulfonylurea herbicide, DPX-T9595 has recently been developed for use in cotton (Strachan *et al.*, 1988). Cotton rapidly metabolizes this sulfonylurea herbicide to nonphytotoxic residues, whereas most of the broadleaf weeds in cotton do not metabolize it (Wittenbach *et al.*, 1988). It promises to fill the niche of a foliar-applied, broadleaf herbicide for use in cotton.

The mechanism of action of the sulfonylurea herbicides has recently been reviewed (Beyer *et al.*, 1988; Blair and Martin, 1988). This group of herbicides initially stops the growth of target plants. Cessation of growth is followed by other symptoms such as chlorosis and reddening (anthocyanin synthesis). After several days, or even weeks, the plant dies. In some plant species, growth is stunted and the leaves turn darker green than normal. Like glyphosate (Roundup®), this group of herbicides is phloem mobile and is readily translocated to metabolic sinks (*i.e.*, growing tissues). Sulfonylurea herbicides apparently have only one important molecular site of action, the enzyme acetolactate synthase (ALS). ALS is necessary in the synthesis of the branched chain amino acids valine, leucine and isoleucine. Thus, as with glyphosate, the growth of the plant stops because proteins cannot be synthesized and metabolic processes are disrupted. Plant cells that cannot produce proteins, die prematurely because cellular maintenance is stopped.

Unlike glyphosate, the sulfonylureas are readily metabolized by many plant species. Differential metabolism has been found to be the basis of selectivity of sulfonylureas in most cases. However, it is possible to produce plants resistant at the site of action (ALS) by selection at the whole plant level (Haughn and Somerville, 1986). Thus, it is reasonable to expect sulfonylurea-resistant weeds to occur in areas in which only sulfonylureas or other ALS-inhibiting herbicides (*e.g.*, imidazolinones) are used to control particular weed species over a period of years.

S-TRIAZINES

Numerous *S*-triazines such as cyanazine (Bladex®), ametryn (Evik®), prometryn (Caparol®) and dipropetryn (Sancap®) are used primarily for preemergence control of weeds in cotton. More is known of the mechanism of action of this class of herbicides than any other.

These relatively lipophilic compounds are readily absorbed by roots and translocated to shoots of all species via the transpirational stream (apoplastic movement) (Ashton and Crafts, 1981). However, foliar absorption is much more limited and translocation from treated leaves is very limited because it is almost exclusively apoplastic (Ashton and Crafts, 1981).

Cotton roots readily absorb simazine (Princep®) and translocate it to leaves (Davis *et al.*, 1959). However, unlike in other species, it accumulates in the terpenoid-filled lysigenous glands of cotton (Davis *et al.*, 1959; Foy, 1964; Sikka and Davis, 1968) (Figure 8) as do other lipophilic herbicides such as pyridazinones

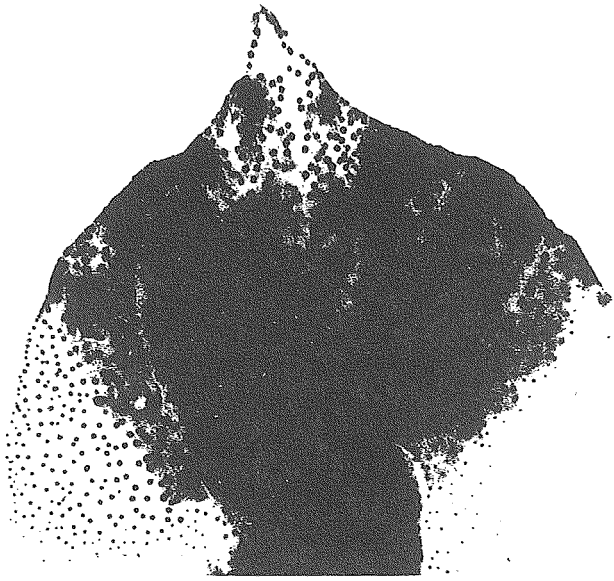

Figure 8. Autoradiogram of a cotton leaf 72 hours after treatment with ^{14}C-ipazine showing accumulation in lysigenous glands. (Foy, 1964.)

and dinitroanilines, as discussed above. In most tolerant species, tolerance to *S*-triazines is due almost entirely to metabolism (Shimabukuro, 1985). However, in cotton tolerance is due to poor translocation, metabolism by *N*-dealkylation and sequestering of the herbicide in the lysigenous glands and seed coat (Sikka and Davis, 1968; Shimabukuro and Swanson, 1970; Rubin and Eshel, 1978).

Although the *S*-triazines can have many and diverse metabolic effects on plants (Ashton and Crafts, 1981), there is no doubt that their primary effect is inhibition of photosynthesis by blocking photosynthetic electron transport (Figure 9). Good evidence of this is provided by the increase in variable fluorescence of chlorophyll in leaves treated with this herbicide class (Figure 10). Light energy absorbed by chlorophyll that cannot be dissipated by photosynthesis, chemical transfer or thermal loss is given off as variable fluorescence in the red part of the spectrum. The more inefficient that chloroplast electron transport is, the higher the level of variable fluorescence. The I_{50}'s for triazine herbicides on certain fluorescence parameters of isolated chloroplasts is as low as 0.1 μM (Brewer *et al.*, 1979).

Photosystem II inhibitors such as the *S*-triazines cause numerous secondary effects by blocking electron flow. The light energy absorbed by chlorophyll is dissipated as fluorescence and as heat; however, a particular form of light-energized chlorophyll (triplet chlorophyll) can transfer energy to molecular oxygen to form singlet oxygen, the same destructive form of oxygen thought to be the cause of *p*-nitrosubstituted diphenyl ether (NDPE) herbicide activity (see above

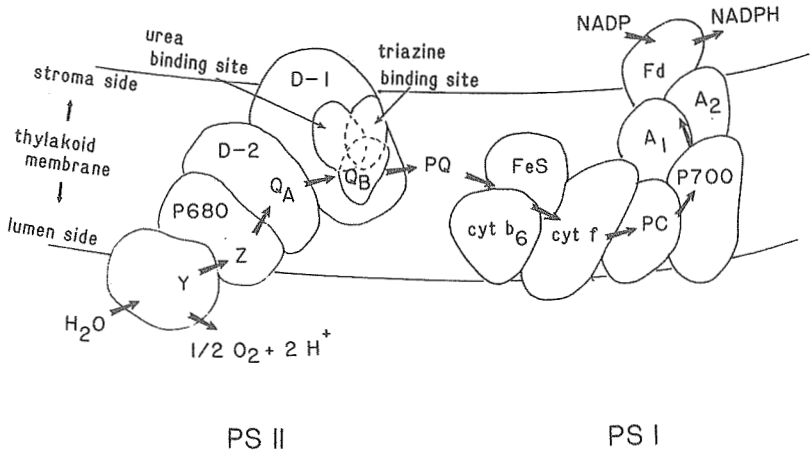

Figure 9. Schematic of components of photosynthetic electron flow (bold arrows) in thylakoid membranes of higher plants and point of inhibition of S-triazine and urea herbicides. P680 = reaction center for PS II, Z = PS II donor that is oxidized by light absorption of chlorophyll and in turn causes oxidation of water by magnesium-containing proteins (Y), D-2 = Quinone A(Q_A)-binding protein, D-1 = Quinone B(Q_B)-binding protein, PQ = plastoquinone, cyt b_6 = cytochrome b_6, FeS = non-heme iron sulfur center, cyt f = f type cytochrome, P700 = PS I reaction center, Fd = ferredoxin.

and Figure 7). The injury is not as rapid as with the NDPEs because the chloroplast is well protected from singlet oxygen by carotenoids and high levels of α-tocopherol (vitamin E). Thus, with *S*-triazines there is usually time for the cessation of carbon fixation and accompanying metabolic disruption to play a role in the deterioration of the plant. Separating singlet oxygen effects from metabolic disruption effects is difficult; however, damage caused by singlet oxygen will be relatively greater as light intensity increases.

The exact location of the blockage is the D-1 quinone-binding protein that is located between the primary quinone electron acceptor of PS II, located on the D-2 protein, and plastoquinone (Renger, 1986) (Figure 9). This location of the blockage in the electron transport chain of the chloroplast by triazines was indicated by use of artificial electron donor and acceptor couples for different components of the electron transport chain (Pfister and Urbach, 1983). With these techniques, most segments of the electron transport system can be studied separately. These results and fluorescence data led to the hypothesis that triazine

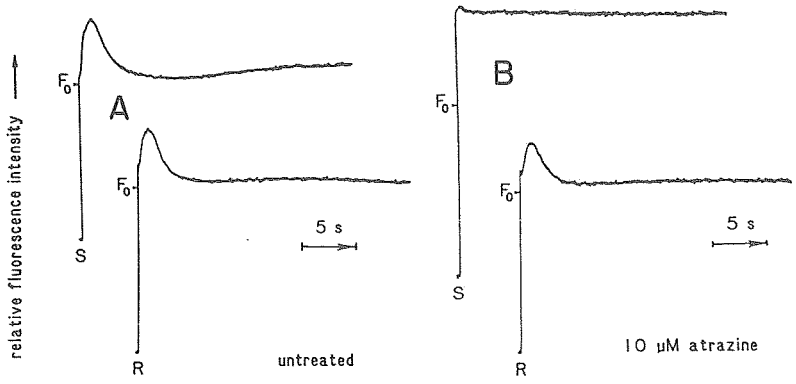

Figure 10. Variable fluorescence of untreated (A) and 10 μM-atrazine-treated (B) triazine-sensitive (S) and resistant (R) biotypes of pigweed (*Amaranthus hybridus*). (Adapted from Vaughn and Duke, 1984.)

herbicides compete for the binding site of the B quinone involved in electron transport between the PSII electron acceptor quinone Q and the plastoquinone pool (Figure 9) (Velthuys, 1981).

This hypothesis was strengthened by discovery of the identity of the quinone B binding protein (now called D-1). Its identity was obtained through the use of a form of radiolabeled atrazine (^{14}C-azido-atrazine) that irreversibly binds to the molecule closest to it when irradiated with ultraviolet light. Using this technique, Pfister *et al.* (1982) found a chloroplast thylakoid membrane protein with a molecular mass of 32 kD strongly labeled with radioactivity (Figure 11). No labeling was found in the thylakoid membranes of triazine-resistant biotypes (Figure 11). This protein is maternally inherited through transmission on the chloroplast genetic code (the plastome), as is resistance to triazine herbicides (Gressel, 1985). Furthermore, there is only a one amino acid difference (serine 264 to glycine) that eliminates triazine binding and confers triazine resistance (Hirschberg *et al.*, 1984). Biotypes with this genetic alteration are generally more than 100-fold less sensitive to triazine herbicides than sensitive biotypes of the same species.

Extensive studies of the molecular biology of D-1 and its role in photosynthesis and plant responses to light have resulted from this work on the mode of action of triazine herbicides. This work also has resulted in a much clearer understanding of other herbicide classes such as the ureas that act at the same site.

Figure 11. Thylakoid proteins of triazine-susceptible and triazine-resistant biotypes of pigweed (*Amaranthus hybridus*) separated by polyacrylamide gel electrophoresis. The lane on the left (labeled A) for each biotype has been stained for protein, and the lane on the right (labeled B) is a autoradiograph of proteins labeled with ^{14}C-azido-atrazine. (Courtesy of K. Pfister, CIBA-GEIGY.)

UREAS

The substituted ureas are among the oldest herbicide classes, introduced in the 1950s. Today, linuron (Lorox®), diuron (Karmex®) and fluometuron (Cotoran®) are used in cotton in the United States. Although structurally different from the *S*-triazines, the substituted ureas share many similarities in their mode of action and modes of tolerance.

Like the triazines, they are used in cotton as preemergence herbicides. In general, the substituted ureas are rapidly absorbed by roots and translocated through the xylem to the leaves (Ashton and Crafts, 1981). Like the triazines, the substituted ureas preferentially accumulate in the lysigenous glands and trichomes of cotton (Strang and Rogers, 1971). Cotton also metabolizes substituted ureas to nonphytotoxic metabolites (Rogers and Funderburk, 1968; Rubin and Eschel, 1971). Nevertheless, cotton is only tolerant, but not resistant, to this herbicide class.

Their mechanism of action is virtually identical to that of the *S*-triazines (see above). The substituted urea herbicides block photosynthetic electron transport at precisely the same point in the system as the *S*-triazines; however, their binding site is not identical to that of the triazines (Pfister, 1983) (Figure 9). Diuron

displaces radiolabeled atrazine on the thylakoid membrane; however, triazine-resistant biotypes of weeds are not resistant to substituted ureas (Fuerst *et al.*, 1986). Thus, the one amino acid alteration in D-1 (Figure 9) that greatly affects triazine binding has virtually no effect on the binding of substituted ureas. No instances of substituted urea herbicide resistance have been reported in higher plants. Alternating substituted urea and *S*-triazines in cotton should retard the occurence of triazine-resistance in this crop.

Although methazole (Probe®) is not a urea, once inside the plant, it is metabolized to the substituted urea DCPMU [1-(3,4-dichlorophenyl)-3-methylurea] (Jones and Foy, 1972). DCPMU is a potent inhibitor of PS II electron transport (Good, 1961). Cotton is tolerant to methazole because, after metabolizing it to DCPMU, DCPMU is further metabolized to the relatively nonphytotoxic DCPU [1-(3,4-dichlorophenyl)urea] (Jones and Foy, 1972).

BIOTECHNOLOGY AND FUTURE DIRECTIONS

HERBICIDE DESIGN

As mentioned in the introduction, understanding mode of action can be useful in the biorational design of herbicides. There is no obvious biochemical characteristic of cotton that points toward herbicide design around a particular biochemical site other than those characteristics that distinguish it from monocots. No strong degradative pathway in cotton has been found that might rapidly degrade a particular class of chemical compounds. The only feature of cotton that appears to lend tolerance to a large number of herbicides is the presence of lysigenous glands filled with lipophilic terpenoids. Sequestration of lipophilic herbicides into these glands has been implicated in the tolerance of cotton to dinitroanilines, *S*-triazines and substituted ureas. Therefore, lipophilic compounds may have a natural advantage over other herbicides for use in cotton. A potential ploy in developing new herbicides for cotton would be to increase the lipophilicity, while retaining activity, of herbicides that cannot currently be used in cotton.

Another ploy with potential for development of new cotton herbicides would be to test the phytotoxins produced by pathogens of weeds in cotton. Often, phytotoxins produced by pathogens are highly active with excellent selectivity (Duke, 1986a,b). Naturally-occuring phytotoxins are being actively screened for herbicidal activity by industry and are being patented as herbicides. These compounds often attack previously unexploited sites of action (Duke, 1986a). To date, however, none of these new chemistries have been reported to have potential as cotton herbicides.

ENGINEERING HERBICIDE RESISTANCE INTO COTTON

Cotton is an ideal prospect for genetically engineering herbicide resistance into a crop. It is third, behind corn and soybean, in herbicide usage in the United States (McWhorter *et al.*, 1985). However, unlike soybean, there are no broad-

leaf herbicides to which it is resistant or highly tolerant when used postemergence. Thus, engineering resistance in cotton to a herbicide to which a company has sole proprietary rights would fill a much-needed gap in weed control in cotton and, accordingly, should be highly profitable. However, if there is significant cross-resistance of the genetically engineered cotton to other herbicides produced by other companies, such a venture might not be profitable. This aspect of engineering herbicide resistance into cotton may limit interest by industry. Therefore, some of this type of research might best be conducted by public laboratories in which the profit motive is not a concern.

From an industry standpoint, genetically engineering resistance to a herbicide like glyphosate (Roundup®) into cotton could be a high return venture. No other herbicides are known to attack the site of action of this herbicide and no cases of resistance in higher plants have arisen. Thus, one over-the-top application of glyphosate to a field of resistant cotton should eliminate all weeds—broadleaves and grasses; annuals and perennials. Bioengineering has already produced glyphosate-resistant higher plants by several methods (Duke, 1988). One method involves transfer of a glyphosate-insensitive form of EPSP synthase (see glyphosate section above) from a bacterium to higher plants (Figure 12); the other entails increasing the number of copies of the EPSP synthase gene (gene amplification) in the plant so that significantly more herbicide is required to completely block the shikimic acid pathway. Another approach that theoretically should work would be to transfer the genes for metabolism of glyphosate from microorganisms to higher plants.

The latter approach does not require a knowledge of the mode of action of the herbicide. The other approaches require that the molecular site of action of the herbicide be understood. If the herbicide acts at more than one molecular site, genetically engineering a resistant plant by manipulation of the sites of action becomes enormously complicated. Thus, a thorough physiological and biochemical determination of the complete mode of action of a herbicide, beginning from the molecular site, should be completed before vast resources are committed to genetically engineering resistance.

A potential problem in genetically engineering resistance of crops to highly translocatable herbicides like glyphosate is that high levels of the herbicide can accumulate in terminal metabolic sinks such as fruits. High herbicide levels in these plant tissues may overcome resistance to the herbicide, causing the plant to abort its fruit or to produce abnormal fruit. If the degree of resistance is high enough to prevent aborted or abnormal fruit, unacceptable herbicide residues in the fruit may pose a problem. These problems should be most severe with herbicides that are not readily metabolized (*e.g.* glyphosate). Accumulation of glyphosate in the fruit may not be important with cotton because of the nature of the principal harvestable products—lint and oil. Because glyphosate is highly water soluble, it may not coextract with cottonseed oil. However, it could reach undesirable levels in cottonseed meal.

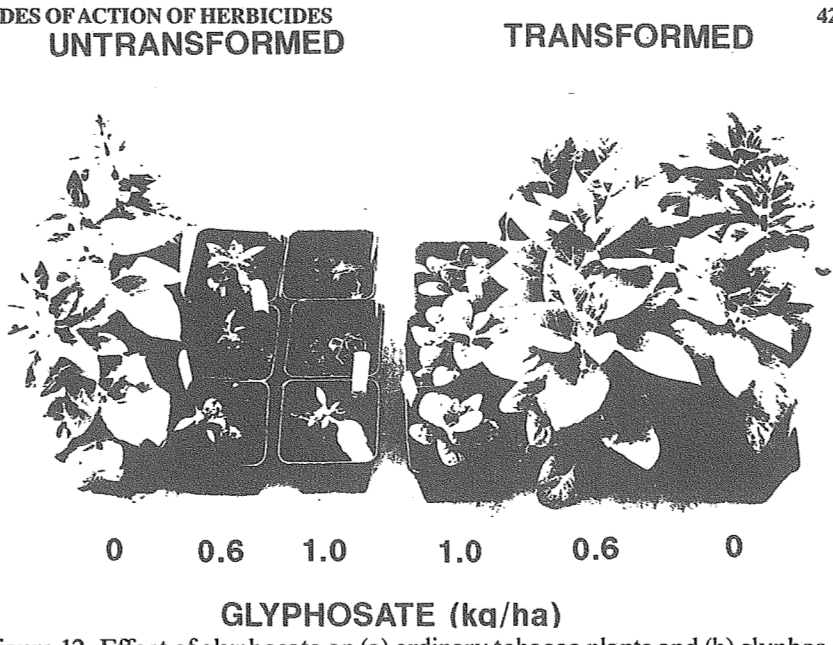

Figure 12. Effect of glyphosate on (a) ordinary tobacco plants and (b) glyphosate-tolerant tobacco plants transformed with the EPSP synthase from glyphosate-resistant *Salmonella typhimurium*. (Courtesy of Luca Comai of Calgene.)

Bromoxynil (Buctril®, Brominal®)-resistant cotton has been produced by transferring the gene from the soil bacterium *Klebsiella ozaenae* that codes for a nitrilase which detoxifies the herbicide (Stalker *et al.*, 1988). This genetically engineered cotton has been field tested by Calgene, Inc. and has potential for adding bromoxynil to the arsenal of herbicides available to cotton farmers.

SUMMARY

There is no general mode of action of cotton herbicides. The herbicides used in cotton attack a large number of biochemical and physiological sites that are summarized in Table 1. Since there is no suitable broadleaf herbicide for over-the-top application in cotton, this problem is a logical focus for future herbicide discovery efforts. The new tools of molecular biology and genetic engineering should be crucial in solving this problem. However, without a thorough knowledge of the mode of action of the herbicide being researched, significant progress with either biorational or genetic engineering approaches is not likely to improve weed control in cotton with herbicides.

Table 1. Summary of the modes of action of cotton herbicides.

Herbicide class	Mode of action
Arsenicals	Inhibition of carbon fixation by covalent bonding of arsenomethane to SH-containing enzymes
Aryloxyphenoxy alkanoic acids	Inhibition of lipid synthesis
Chlorinated aliphatic acids	Unknown, perhaps pantothenic acid synthesis
Chloroacetamides	Unknown, perhaps alkylation of proteins or lipid synthesis
Cineoles	Unknown
Cyclohexanediones	Inhibition of lipid synthesis
DCPA	Unknown, perhaps interference with tubulin function
Dinitroanilines	Interference with tubulin function
Glyphosate	Blockage of the shikimic acid pathway
Isoxazolidinones	Inhibition of the diterpenoid synthesis
p-nitrosubstituted diphenyl ethers	Inhibition of the porphyrin synthesis pathway, resulting in buildup of photodynamic pigments
Pyridazinones	Inhibition of carotenoid synthesis
Sulfonylureas	Inhibition of branched chain amino acid synthesis
S-triazines	Inhibition of photosynthetic electron transport at the Q_B site on D-1.
Ureas	Inhibition of photosynthetic electron transport at the Q_B site on D-1.

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